INFLUENCE OF DOPAMINE RECEPTOR ACTIVATION ON NEUROSECRETION
FROM PERFUSED RAT HYPOTHALAMO-NEUROHYPOPHYSIAL EXPLANTS

by

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ABSTRACT

The hypothalamic supraoptic nucleus (SON) contains both vasopressin-secreting (AVP) and oxytocin-secreting (OT) magnocellular neurons which release their respective hormones from the posterior pituitary gland into the systemic circulation. A direct relationship has been demonstrated in earlier physiological studies between the excitability of SON neurons and hormone release from the neurohypophysis. Catecholamines have been shown to activate SON neurons, thereby increasing the action potential frequency to the neural lobe. The present study examined the ability of catecholamines to evoke release of vasopressin and/or oxytocin from the neural lobe of an intraarterially perfused preparation of rat hypothalamo-neurohypophysis.

Experiments were carried out on male Long-Evans rats. Drugs (noradrenaline, dopamine, D1 and D2 dopamine receptor agonists) were delivered both into the perfusion medium and directly over the SON. Fractions of effluent medium were collected from the area of the neurohypophysis and tested for hormone release using a selective radioimmunoassay. Noradrenaline (NA) in concentrations of $10^{-5}$ to $10^{-4}$ M
stimulated AVP release from neural lobe. The \( \alpha \) adrenergic receptor antagonist, prazosin (50 nM in perfusion experiments; 1\( \mu \)M in local injection experiments) blocked the noradrenaline-stimulated release. Dopamine (10\(^{-4}\) to 10\(^{-1}\) M), D1 (SKF 38393, 0.5 mM) and D2 (QNP 0.5 mM) dopamine receptor agonists also increased the release of AVP. In the presence of prazosin, DA, QNP and SKF 38393 still evoked AVP release. Therefore, it is proposed that both noradrenaline and dopamine stimulate the release of AVP in this preparation; the responses to dopamine appear to be mediated by both D1 and D2 dopamine receptors, presumably located at the level of the cell somata/dendrites, possibly also on axon terminals in the neural lobe.
Résumé

Le noyau supraoptique (SON) contient les neurones magnocellulaires à vasopressine (AVP) et à oxytocine (OT) qui libèrent leur contenu neurosécrétoire dans la circulation systémique après l'avoir emmagasiné dans la neurohypophyse.

Des études physiologiques ont déjà démontré une relation directe entre le niveau d'excitation des neurones du noyau supraoptique et les niveaux d'hormone libérée par la neurohypophyse. Il a déjà été démontré que les catécholamines stimulent les neurones du noyau supraoptique augmentant ainsi la fréquence des potentiels d'action détectés au niveau du lobe neural. Dans notre étude, nous analysons l'effet des catécholamines sur la libération de vasopressine et/ou d'oxytocine du lobe neural du rat en utilisant un bloc hypothalamo-hypophysaire perfusé in vitro par voie artérielle.

Les expériences sont effectuées sur des rats mâles Long-Evans. Les substances suivantes: noradrenaline, dopamine, agonistes (or antagonistes) des récepteurs dopaminergiques D1 et D2 sont administrées dans le milieu de perfusion ou directement sur le noyau supraoptique. L'effluent est collecté
tout près de la neurohypophyse dans des fractions dont le contenu en vasopressine est ensuite mesuré par essai radioimmunologique. Nous avons observé que la noradrénaline (NA) aux concentrations de 10⁻⁷ à 10⁻⁴ M stimulait la libération de vasopressine du lobe neural. Cette libération de la vasopressine est stimulée par la prazosine, un antagoniste du récepteur alpha-1 adrénergique à des concentrations de 50 mM en perfusion ou 1 mM en injection locale. La dopamine, 10⁻¹ à 10⁻³ M, les agonistes du récepteur dopaminergique D₁ (SKF 38393; 0.5 mM) et du récepteur D₂ (QNP; 0.5 mM) augment aussi la libération de vasopressine.

Nous proposons donc que la dopamine, tout comme la noradrénaline, stimule la libération de la vasopressine dans notre préparation. Les réponses à la dopamine semblent médiane par les récepteurs dopaminergiques D₁ et D₂ que l'on croit localisés à la surface du soma et des dendrites, possiblement aussi sur les terminaisons axonales du lobe neural.
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1. ABBREVIATIONS

Å: angstrom, 10^{-7} mm.
A1: group of noradrenergic cells in the ventrolateral medulla.
A2: group of noradrenergic cells in the dorsomedial medulla.
A6: group of noradrenergic cells in the lateral part of the pontine central gray.
Ab: antibody.
ACSF: artificial cerebrospinal fluid.
AII: angiotensin II.
AV3V: anteroventral region of the third ventricle.
AVP: arginine vasopressin.
C1: group of adrenergic neurons in the ventrolateral medulla.
C2: group of adrenergic neurons in the dorsomedial medulla.
C3: group of adrenergic neurons in the dorsomedial medulla.
CA: catecholamine.
cAMP: cyclic adenosine monophosphate
CAT: choline acetyltransferase.
CCK: cholecystokinin.
cNTS: caudal nucleus tractus solitarius.
CO₂: carbon dioxide.
cpm: counts per minute.
CSF: cerebrospinal fluid.
cVLM: caudal ventrolateral medulla.
DA: dopamine.
DBB: diagonal band of broca.
DMSO: dimethyl sulfoxide.
EDTA: ethylenediaminetetraacetic acid.
g: gram.
GABA: gamma-amino-butyric acid.
GAD: glutamic acid decarboxylase.
HRP: horseradish peroxidase.
i.c.v.: intracerebroventricular injection.
M: molar.
μl: microliter.
μM: micromole.
μm: micrometer.
mg: milligram.
ml: milliliter.
mm: millimeter.
mM: micromole.
MNC: magnocellular neurons.
MnPO: median preoptic nucleus.
mNTS: medial subnucleus of the nucleus tractus solitarius.
MPO: medial preoptic area.
M.W.: molecular weight.
NA: noradrenaline.
nM: nanomole.
NIL: neurointermediate lobe.
NL: neural lobe.
NSG: neurosecretory granules.
NTS: nucleus tractus solitarius.
6-OHDA: 6-hydroxy-dopamine.
OT: oxytocin.
OVLT: organum vasculosum lamina terminalis.
PEG: polyethylene glycol.
pg: picogram.
pH: hydrogen ion potential.
PHA-L: phaseolus vulgaris leucoagglutinin.
PVPO: periventricular preoptic area.
PVN: paraventricular nucleus.
PVNmc: magnocellular neurons in the PVN.
PVNPC: parvocellular neurons in the PVN.
QNP: quinpirol hydrochloride.
RIA: radioimmunoassay.
r.p.m.: revolutions per minute
SFO: subfornical organ.
SON: supraoptic nucleus.
2. INTRODUCTION

2.1. The Concept of Neurosecretion

The term "neurosecretion" refers in the classical definition to the release of a hormone (transmitter) from the terminal of a neuron into a vascular compartment. A more comprehensive definition is the release at a somatic, dendritic or axon site of any product synthesized by a neuron, be it transmitter or hormone. The mechanisms of neurosecretion include the stimulus that drives the electrical and biochemical components of neurosecretion. The latter includes ribosomal synthesis of a neuropeptide, its packaging into neurosecretory granules and their rapid transport through axons. The former includes stimulus-secretion coupling and exocytosis of the granule contents at the axon terminal (Brownstein et al., 1980; Silverman and Zimmerman, 1983). This thesis will deal with a neurotransmitter, dopamine, and its ability to evoke the neurosecretion of vasopressin from the isolated rat hypothalamic-pituitary explant maintained in vitro.
2.2. The Classical Mammalian Magnocellular Neurosecretory System

The term "neurosecretory neurons" describes a class of cells which have the morphological and functional properties of both neurons and endocrine gland cells (Brownstein et al., 1980). Thus, these neurosecretory neurons have a dual character: one is neuronal, since these cells have somata and dendrites to receive signals, and axonal processes to transmit electrical signals to terminals; the other is hormonal since they produce products that are released from axon terminals at a neurohaemal junction and are transported in the circulation to target organs throughout the body. In addition, an interesting recent discussion has arisen related to possible local release of products from their dendrites in the hypothalamus (Pow and Morris, 1989).

The classical magnocellular neurosecretory system in mammals comprises two general groups of neurons in the hypothalamus whose axons terminate in the posterior lobe of the pituitary gland (Peterson, 1966; Sherlock et al., 1975; Silverman and Zimmerman, 1983): 1) the large neurons (20–35 μm in diameter) of the supraoptic (SON) and paraventricular nuclei (PVN), 2) the accessory magnocellular cell groups, located along the larger hypothalamic arteries traversing the area between the SON and the PVN. The latter include a rostral
paraventricular nucleus, anterior and posterior fornical nuclei, the nucleus circularis, the nucleus of the medial forebrain bundle, an irregular string of cells that extend laterally and ventrally from the PVN to the SON, and some scattered magnocellular cells in the preoptic region, the zona incerta and the bed nucleus of the stria terminalis (Kelly and Swanson, 1980).

2.3. The Supraoptic and Paraventricular Nuclei of the Hypothalamus

2.3.1. Organization of the SON and PVN

The paired SON are elongated groupings of magnocellular neurons located on the ventral surface of the brain, lying along blood vessels penetrating the optic chiasm and optic nerve (Swanson and Sawchenko, 1983). Each SON in the rat consists of between 4,400 (Léránth et al., 1975) and 7,000 cells (Bodian and Maren, 1951). Rostrally, the SON forms a thin layer of cells along the ventral surface of the diencephalon. Dorsally, the nucleus is bordered by the lateral hypothalamus and medial forebrain bundle. Caudally, the SON neurons form a narrow vertically oriented band of cells along the proximal portion of the optic tract where it penetrates...
the temporal lobe. Virtually all SON neurons project their axons to the neurohypophysis (see below).

The PVN contains both magnocellular (PVNmc) and parvocellular (PVNpc) neurons (Krieg, 1932; Swanson and Sawchenko, 1983). The magnocellular PVN consists of three distinct subdivisions. The anterior magnocellular part is located ventromedial to the descending column of the fornix as it enters the hypothalamus from the septum. The medial magnocellular subdivision is embedded within the anterior periventricular nucleus and lies just caudal to the anterior magnocellular part (Swanson and Sawchenko, 1983). Collectively these parts contain about 400-600 cells (Rhodes et al., 1981; Sawchenko and Swanson, 1982b). The posterior magnocellular part is located in ventromedial regions of the PVN immediately caudal to the medial magnocellular part of the nucleus and expands dorsolaterally to form a compact cell mass at the lateral margin of the PVN (Swanson and Sawchenko, 1983). This is the largest of the magnocellular subdivisions and contains 1300 to 2000 cells (Bodian and Maren, 1951; Bandaranayake, 1971).

The parvocellular division of PVN has at least five distinct components: the anterior, medial, dorsal, and lateral parvocellular and periventricular parts. These contain a heterogeneus group of neurons. Neurons in the medial and
periventricular parts project to the median eminence portal plexus and are involved in regulation of anterior pituitary hormone secretion. Those in the dorsal part project axons to brainstem and spinal autonomic centres (Sawchenko and Swanson, 1982b).

Studies utilizing retrograde transporting techniques have shown that almost all SON cells (Sherlock et al., 1975) and a large majority of PVNmc cells (Sherlock et al., 1975; Kelly and Swanson, 1980; Armstrong and Hatton, 1980, Armstrong et al., 1980; Wiegand and Price, 1980) are labelled following an injection of horseradish peroxidase (HRP) into the neural lobe, indicating that they send an axon to the neural lobe. Within the PVN, cell bodies retrogradely labelled from the neural lobe are concentrated mostly in the medial and lateral magnocellular parts. A few cells in the parvocellular subdivisions of the PVN are also labelled following an injection of HRP into the neurohypophysis (Armstrong et al., 1980; Wiegand and Price, 1980).

2.3.2. Morphology

2.3.2.1. SON light microscopic features
The SON and PVNmc are easily recognized in standard Nissl preparations as discrete aggregates of closely packed and intensely stained perikarya (Swanson and Sawchenko, 1983).

The magnocellular SON and PVN neurosecretory neurons are large (20-35 μm in diameter) (Sofroniew and Glasmann, 1981; Dyball and Garten, 1988) oval cells. In the SON, they usually have one to five primary dendrites that do not branch extensively. At least one of these is directed ventrally (Silverman and Zimmerman, 1983; Swanson and Sawchenko, 1983; Dyball and Garten, 1988) to the ventral glial lamina, regardless of whether the somata is located ventrally or dorsally in the nucleus (Armstrong et al., 1982a; Randle et al., 1986c). Other dendrites extend medially along the optic tract (Randle et al., 1986c, Smithson et al., 1989) or dorsolaterally towards the temporal lobe (Oldfield et al., 1985). A single axon directed dorsomedially emanates either from a proximal dendrite or from the cell body itself (Armstrong et al., 1982a; 1982b). Axons may extend short collateral branches along their course (Silverman and Zimmerman, 1983; Sofroniew et al., 1988) but do not have extensive collaterals; the main axons form the supraopticohypophysial tract (Swanson and Sawchenko, 1983). The axons of the SON neurons are beaded in appearance due to numerous rounded enlargements or varicosities (Sofroniew and Glasmann, 1981).
2.3.2.2. PVNmc light microscopic features

The magnocellular neurons in the PVN are quite similar in morphology to those in the SON. While a majority of dendritic processes remain within the morphological boundaries of the nucleus, some dendrites in the more caudal groups extend laterally away from the nucleus (Armstrong et al., 1980). The axons of magnocellular neurons leave the nucleus laterally and sweep over the SON; others course ventrolaterally at the level above or below the fornix, and then posteroventromedially to join the supraopticohypophysial tract and course through the zona interna of the median eminence (Van Den Pol, 1982).

2.3.3. Ultrastructure

The somata of magnocellular neurosecretory neurons contain a large, eccentrically located nucleus with one or more nucleoli, mitochondria, rough endoplasmic reticulum, Golgi apparatus, lysosomes and Nissl substance. Their dendrites contain neurofilaments. Their axons contain neurofibrils and their axon terminals contain 2 types of vesicles: one is 500-600 Å in diameter; the other is 1,200-1,500 Å and contains neurosecretory material. These larger granules contain and store the vasopressin (AVP) and oxytocin (OT) which, along with their respective neurophysins, are synthesized from
larger precursor molecules in the magnocellular neuron (Brownstein et al., 1980)

Three kinds of synapses (axo-dendritic, axo-somatic, and axo-axonic) have been observed in the SON (Rechardt, 1969; Priymak and Hajós, 1970; Léránth et al., 1975; Silverman et al., 1980) and PVN (Van Den Pol, 1982; Silverman and Zimmerman, 1983). In a quantitative electron microscopical study, Léránth et al. (1975) observed that each SON neuron receives about 600 synapses distributed on its soma, dendrites, dendritic spines and axon. Two thirds of the inputs to SON neurons are presumed to originate from within or near the nucleus since this number of synapses do not undergo degeneration following knife cuts that isolate the SON from surrounding tissues (Záborsky et al., 1975).

In the PVN, synapses are found on shafts and spines of dendrites, on perikarya and somatic appendages, and invaginated into the soma. Both dendrites and axons with large neurosecretory vesicles immunostained for neurophysin are found postsynaptic to other axons. In at least one instance, axon terminals containing neurosecretory product has been reported within the SON (Theodosis, 1985).

2.3.4. Immunocytochemistry
The availability of sensitive and specific immunocytochemical techniques provided a direct proof of the central distribution of neuropeptides throughout the animal kingdom. Before the application of immunocytochemical techniques to the magnocellular neurosecretory system in the mid 1970s, it was not clear whether oxytocin and vasopressin were localized within the same or separate populations of cells, and which hormone was localized preferentially in the SON. At one time it was thought that vasopressin was synthesized primarily in the SON, and oxytocin in the PVN (Swanson and Sawchenko, 1983). Using immunocytochemical staining for vasopressin and oxytocin, it was observed that separate populations of SON and PVNmc cells synthesize these two peptides (Vandesande and Dierickx, 1975; Swaab et al., 1975a; 1975b; Sokol et al., 1976).

Most (over 85%) cells in the SON are immunoreactive for vasopressin or oxytocin and their associated neurophysins (Vandesande and Dierickx, 1975; Rhodes et al., 1981). No distinct morphological differences between the two kinds of neurosecretory cells could be observed (Vandesande and Dierickx, 1975) except for location. In the SON of the rat, oxytocin cells are concentrated anterodorsally and vasopressin cells concentrated posterodorsally (Swaab et al., 1975b; Vandesande and Dierickx, 1975; Sokol et al., 1976; Rhodes et al., 1981; Sawchenko and Swanson, 1982a). The ratio of
vasopressin cells to oxytocin cells in the SON varies from 1:1 (Vandesande and Dierickx, 1975) to 1.6:1 (Swaab et al. 1975a; 1975b).

In the PVN, the organization of vasopressin and oxytocin-immunoreactive neurons is no less distinctive. The anterior and medial magnocellular parts consist almost exclusively of oxytocin-stained cells. In the posterior magnocellular part of the PVN, vasopressin-stained cells are concentrated posterodorsolaterally, oxytocin-stained cells are localized anteroventromedially (Rhodes et al., 1981; Sawchenko and Swanson, 1982b).

Several other peptides have been observed histochemically to colocalize in magnocellular neurosecretory neurons (see Meister et al., 1990 for review). Two groups of peptides deserve special mention:

1) the opiate peptides: The hypothalamus is rich in both opiate binding sites (Simantov and Snyder, 1977) and in opioid peptides. Enkephalin immunoreactivity has been demonstrated in the SON and PVN (Sar et al., 1978; Rossier et al., 1979; Finley et al., 1981; Sawchenko and Swanson, 1982b; Swanson and Sawchenko, 1983). In the rat neurohypophysis, Martin and Voigt (1981) have suggested that met-enkephalin is always found within oxytocin terminals (also Martin et al., 1983) and leu
enkephalin usually, but not always, within vasopressin terminals. Another opioid peptide present within all vasopressin neurons is dynorphin (Watson et al., 1980; 1981; 1982; Weber et al., 1982). A possible role of opiates (Aziz et al., 1981; Iversen et al., 1980; Haldar and Sawyer, 1978) and endogenous opioid peptides (Bisset et al., 1978; Weitzman et al., 1977; Miller, 1980; Rossier et al., 1979; Clarke et al., 1979a; Iversen et al., 1980; Summy-Long et al., 1981; Bondy et al., 1988; Yamada et al., 1988) has been shown on the release of vasopressin and oxytocin. Opioid peptides generally decrease the release of both vasopressin (Summy-Long et al., 1981; Aziz et al., 1981) and oxytocin (Haldar and Sawyer, 1978; Clarke et al., 1979a; Yamada et al., 1988; Bondy et al., 1988) and also inhibit the activity of vasopressin secreting neurons in the SON (Clarke et al., 1980). This inhibitory effect of opioid peptides can be blocked by an opioid antagonist, naloxone (Bicknell et al., 1985; Hartman et al., 1987; Bondy et al., 1988; Yamada et al., 1989). Some (conflicting) reports indicate that opioid peptides have a stimulatory effect (Aziz et al., 1981; Weitzman et al., 1977) or no effect (Bicknell et al., 1985) on hormone release.

2) the gastrin cholecystokinin family: Cholecystokinin coexists within many oxytocin neurons of the SON and PVNmc (Vanderhaegen et al., 1980; Martin et al., 1983). Cholecystokinin is reported to directly depolarize rat SON
neurosecretory neurons (Jarvis, et al., 1988) and to provoke hormone release from perfused hypothalamic explants (Jarvis, et al., 1989). It also stimulates neurohypophyseal hormone secretion in rats (Verbalis et al., 1986; Bondy et al., 1989; Jarvis et al., 1989), monkeys (Verbalis et al., 1987) and humans (Miaskiewicz et al., 1989) when administered systemically. In the rat SON, this response is correlated with a selective enhancement of activity in oxytocinergic neurons (Renaud et al., 1987).

2.4. Cell Biology of AVP and OT-Synthesizing Neurons

Vasopressin and oxytocin are structurally related nonapeptides with molecular weights of 1084 and 1007 daltons, respectively and the following structure

**Vasopressin:**

\[
\begin{align*}
\text{O} & \quad \text{NH}_2 - \text{Cys} - \text{Tyr} - \text{Phe} \\
\text{NH}_2 - \text{C} - \text{Gly} - \text{Arg} - \text{Pro} - \text{Cys} - \text{Asn} - \text{Gln}
\end{align*}
\]

**Oxytocin:**

\[
\begin{align*}
\text{O} & \quad \text{NH}_2 - \text{Cys} - \text{Tyr} - \text{Ile} \\
\text{NH}_2 - \text{C} - \text{Gly} - \text{Leu} - \text{Pro} - \text{Cys} - \text{Asn} - \text{Gln}
\end{align*}
\]
The major physiological roles of vasopressin are antidiuretic action (regulation of water balance in animals) and vasopressor action (contraction of arterioles); those of oxytocin are contraction of the uterus (contraction of smooth muscle cells) and stimulation of milk ejection in the lactating mammary gland (contraction of myoepithelial cells).

Vasopressin and oxytocin are derived from prepropressophysin and preprooxytocin respectively, large prohormones synthesized in the somata of magnocellular neurosecretory neurons, and then packaged into secretory granules (via the Golgi apparatus). In neurosecretory cells the hormone precursors are cleaved during axonal transport. Thus the granules will contain both the hormones themselves and other cleavage products including carrier proteins (i.e. neurophysins) and residual bits of precursor. These granules release their products via an exocytotic process into the systemic circulation in response to electrical or chemical depolarization of the axon terminals (Brownstein et al., 1980; Sklar and Schrier, 1983).

2.4.1. Characterization of neurohypophysial prohormones

Although it was proposed many years ago by Sachs and Takabatake (1964), definitive proof of preprohormones for
vasopressin and the oxytocin is relatively recent. This has been shown by protein chemical methods following \[^{35}S\] Cys incorporation in vivo (Russell et al., 1980), also by immunological (Schmale and Richter, 1981a) and tryptic fingerprint (Schmale and Richter, 1981b) analysis, and by recombinant DNA techniques (Land et al., 1982).

2.4.1.1. Preprovasopressin (Prepropressophysin)

The vasopressin/neurophysin precursor is a glycoprotein (M.W. about 20,000 daltons). It contains a 19 amino acid "signal peptide" followed by vasopressin, on the N-terminus, a neurophysin in the middle, and a glycopeptide of 39 amino acids on the C-terminus (Land et al., 1982; Brownstein, 1983). The primary structures of the rat, pig, bovine, and human preprovasopressin are known to have essentially the same structural feature (Land et al., 1982; Ruppert et al., 1984; Sausville et al., 1985; Schmale et al., 1987).

2.4.1.2. Preprooxytocin (Prooxyphysin)

The oxytocin/neurophysin precursor is highly homologous to the preprovasopressin except for the absence of a glycoprotein at the C-terminus (Brownstein, 1983; Land et al.,
1983). The molecular weight is 17,000 daltons (Land et al., 1982).

2.4.2. Processing of AV' and OT

The preprovasopressin and preprooxytocin are synthesized by a ribosomal mechanism on the rough endoplasmic reticulum (Brownstein et al., 1980; Brownstein, 1983). The prohormones make their way next to the Golgi apparatus where the preprovasopressin probably undergoes distal glycosylation. Then they are packaged into secretory vesicles along with the enzymes responsible for their posttranslational processing. The precursors are cleaved enzymatically by removal of the N-terminal signal peptide and, in the case of vasopressin, the C-terminal glycoprotein (Land et al., 1982). This happens at synthesis or immediately following synthesis within the endoplasmic reticulum (Kreil, 1981). Thus the provasopressin and prooxytocin are composed of vasopressin and oxytocin attached to their respective neurophysin by the tripeptide Gly-Lys-Arg (Land et al., 1982; 1983).

2.4.3. Storage and transport
Following synthesis on ribosomes in the somata of the magnocellular neurosecretory cell, the prohormones are packaged in neurosecretory granules. Vasopressin and oxytocin are bound to different proteins, neurophysin II and I, respectively. Therefore, the granules contain three principal products: the neurohypophysial hormones (AVP or OT), carrier proteins (neurophysin II or neurophysin I), and residual bits of precursor (Brownstein et al., 1980). Granules are transported by axoplasmic transports at a rate estimated to be higher than 200 mm per day (Brownstein et al., 1980). The neurosecretory granules (1,000 - 3,000 Å) are the major storage sites of vasopressin and oxytocin. Most of them are found in the nerve terminals although some are found throughout the neuron (Berson and Yalow, 1973 in chapter 10).

2.4.4. Secretion

The secretory granules appear to be released by an exocytotic process when the nerve endings are depolarized. This process involves a calcium-dependent fusion of the granules with the nerve terminal membrane followed by opening of the granules and release of their contents (Brownstein et al., 1980; Brownstein 1983).
2.5. The Relationship of Neurosecretory Neuron Activity and Hormone Release

The electrical activity of magnocellular neurosecretory cells changes according to different physiological conditions, and the amount of hormone released from the neurohypophysis is proportionate to the level of activity of neurosecretory neurons. When the activity of the neurons is increased by electrical stimulation (Dutton and Dyball, 1970; Dreifuss et al., 1971; Wolfe and Gainer, 1986; Hobbach et al., 1988) or by chemical stimulation (Armstrong et al., 1986; Randle et al., 1986), release of AVP and OT is also increased. Moreover, the stimulus pattern is important. In vitro studies reveal that at a given mean stimulation frequency of the isolated neurohypophysis, hormone release is greater when the stimuli are clustered into bursts than when applied with a constant interstimulus interval (Dutton and Dyball, 1970; Bicknell and Leng, 1981; Bicknell et al., 1982; Bicknell, 1988). Hence, the amount of AVP or OT secreted by each action potential is modulated by the same pattern of electrical activity that can be recorded during in vivo extracellular measurements in rats (see Lincoln and Wakerley, 1975 for OT; see Poulain and Wakerley, 1982 for AVP).

2.6. Connections of Neurosecretory Neurons
2.6.1. Efferent connections

As mentioned above, the SON and PVNmc project massively to the neural lobe of the pituitary via predominantly unmyelinated axons that form the supraoptico-hypophysial tract. In general, the axons of magnocellular neurons do not have obvious axon collaterals based on their visualization following intracellular lucifer yellow injection (Randle et al., 1986c). There are rare magnocellular neurons that apparently have axons which innervate both the neural lobe and the lower brainstem (Zerihun and Harris, 1983).

2.6.2. Afferent connections

2.6.2.1. Chemically defined afferents

2.6.2.1.1. Noradrenergic and adrenergic afferents

Carlsson et al. (1962) and Fuxe (1965) used histofluorescence to show that the SON and PVN contain one of the most dense catecholamine innervations in the brain. Andén et al. (1966a; 1966b) initially demonstrated that the cell bodies of these catecholaminergic nerve endings were in the pontine and medullary regions of the brain stem. Studies utilizing neuroanatomical anterograde tracer methods (Sakamoto
et al., 1978; Swanson and Hartman, 1980; Berk and Finkelstein, 1981) and a combination of retrograde axonal transport and immunohistochemistry (Sawchenko and Swanson, 1981; 1982a; Sawchenko et al., 1985) indicate that three cell groups provide almost all of the noradrenergic and adrenergic fibers to the hypothalamus, including the SON and PVN. These include: the Al (noradrenergic) and C1 (adrenergic) cell groups of the ventrolateral medulla, which lie just dorsal to the lateral reticular nucleus; the A2, the C2 and the C3 cell groups of the dorsomedial medulla which are located in a relatively discrete aspect of the medial part of the nucleus of the solitary tract (NTS); the A6 cell group (the locus coeruleus) in the lateral part of the pontine central gray (Swanson and Sawchenko, 1983; Cunningham and Sawchenko, 1988; Sawchenko and Bohn, 1989; Cunningham et al., 1990).

Recently, new information pertinent to the organization, distribution, and morphology of noradrenergic inputs to the SON and PVN has come from the use of a plant lectin, phaseolus vulgaris leucoagglutinin (PHA-L) as an anterogradely transported tracer. Cunningham and Sawchenko (1988) pointed out that both the SON and the PVN receive input from both the A1 and A2 regions. The A1 projection is almost exclusively to the parts of the SON and PVNmc that contain vasopressinergic neurons. The A2 input is comparatively modest with no obvious preferential distribution to vasopressinergic or oxytocinergic.
neurons. Although there is little evidence for a projection from the A6 region to the SON, the PVN does receive A6 noradrenergic afferents, largely restricted to the medial parvicellular division (Cunningham and Sawchenko 1988).

In the SON, noradrenergic fibers are most dense in the ventral region which contains the majority of vasopressinergic neurons (McNeill and Sladek, 1980; Sawchenko and Swanson, 1982a; McKellar and Loewy, 1982; Alonso and Assenmacher, 1984; Decavel et al., 1987). At the ultrastructural level, axosomatic noradrenergic contacts are mostly found in the ventral part of the SON, with only a small number in the dorsal region (Alonso and Assenmacher, 1984; McNeill and Sladek, 1980). It should be pointed out however that a noradrenaline input onto oxytocinergic cells probably exists in the SON, since noradrenergic fibers can also be seen in the anterodorsal region which contains mostly oxytocinergic neurons (McNeill and Sladek, 1980; Decavel et al., 1987). Recall that most SON cells have a dendrite directed into the ventral part of the nucleus. Thus, the ventrally directed dendrites of magnocellular neurons appear to be important to the contact with the noradrenergic inputs (Sawchenko and Swanson, 1982a; Alonso and Assenmacher, 1984).

As mentioned above, the noradrenergic afferents to the magnocellular neurosecretory cells in the SON are derived from
mainly the Al area in the ventrolateral medulla (Swanson and Sawchenko, 1983; Sawchenko and Swanson, 1982a) with a preferential distribution around the vasopressinergic rather than the oxytocinergic neuronal population (McNeill and Sladek, 1980). It is of interest therefore that electrical stimulation of Al area selectively increases the activity of putative vasopressin-secreting SON and PVN neurons, with little or no effect on putative oxytocin-secreting neurons, and that the facilitatory effect is abolished by destruction of the noradrenergic terminal plexus through local application of 6-hydroxydopamine (Day and Renaud, 1984; Day et al., 1984). There remains however some doubt as to the role of noradrenaline in the Al evoked responses noted in post-stimulus histograms (see Day et al., 1990).

Removal of Al noradrenergic innervation of the SON by neurosurgical method causes a postsurgical deficit in excreted AVP levels in twenty-month-old rats in which postsurgical diuresis and decreased urine osmolarity were seen (Phelps et al., 1990). Destruction (range 80-94%) of Al noradrenergic neurons by electrolytic lesion (Head et al., 1987) decreases the amount of AVP released in response to haemorrhage by 83%. Therefore one role of the Al innervation of AVP neurons may be to facilitate their firing (and hence increase AVP release) in response to a hypotensive and/or hypovolemic stimulus (Day and Renaud, 1984).

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Both depressant and excitatory effects of noradrenaline are reported on the release of vasopressin and oxytocin. Both in vivo iontophoretic application (Barker et al., 1971a; 1971b; Moss et al., 1971; 1972b; Arnauld et al., 1983) and in vitro bath application (Sakai et al., 1974) have been reported to decrease the excitability of magnocellular neurosecretory neurons. A suppressant effect on hormone release was also found following intracerebroventricular administration of noradrenaline in vivo (Moos and Richard, 1979; Kimura et al., 1984) or following osmotic stimulation in vitro (Armstrong et al., 1982a). These depressant functions may be mediated through presynaptic α2 and/or postsynaptic β adrenergic receptors (Barker et al., 1971a; Day et al., 1985; Ogata and Matsuo, 1986).

On the other hand, suggestions for a facilitatory action of noradrenaline on AVP release are based on observations following central administration in vivo (Olsson, 1969; Bhargava et al., 1972; Kühn, 1974; Milton and Paterson, 1974; Bridges et al., 1976; Urano and Kobayashi, 1978; Hoffmann, 1979; Willoughby et al., 1987; Leibowitz et al., 1990) as well as exogenous application in vitro (Armstrong et al., 1986; Randle et al., 1986a). Moreover, destruction of central catecholamine structures to deplete noradrenaline in the hypothalamus decreases the release of AVP from neurohypophysis (Milton and Paterson, 1973; Hoffmann et al., 1977; Miller et
al., 1979; Head et al., 1987). The excitation by noradrenaline of both vasopressin- and oxytocin-secretory neurons in the SON in vitro (Armstrong et al., 1986; Randle et al., 1986b; Yamashita et al., 1987; Bourque, 1988) and in vivo (Day et al., 1985) is considered to be mediated through an α₁ adrenoceptor.

2.6.2.2. Dopaminergic input

Three well known neuronal dopaminergic systems, the tuberohypophyseal, the nigrostriatal and the mesolimbic are present in the diencephalon and telencephalon (Ungerstedt, 1971; Moore et al., 1980). In addition, dopamine has also been detected in the SON and PVN by both a fluorometric assay (Metcalf, 1974) and an enzymatic isotopic micromethod assay (Palkovits et al., 1974). In part, this dopamine arises from the incerto-hypothalamic dopamine system, located in the medial zona incerta and the anterior and dorsal hypothalamus (Björklund et al., 1975; Lindvall et al., 1974), and from the periventricular fiber system, distributed in the periventricular areas of the diencephalon and the periventricular gray of the mesencephalon (Lindvall and Björklund. 1974; Lindvall et al., 1974). The use of the combination of glyoxylic acid histochemical fluorescence with various discrete lesions (Björklund et al., 1975) and improved
aldehyde fluorescence histochemistry (Lindvall et al., 1984) have revealed that dopamine fibers do indeed innervate the hypothalamic magnocellular neurosecretory nuclei. Buijs et al. (1984) developed a specific antibody against dopamine and confirmed dopamine immunoreactive terminals synapsing directly on magnocellular neurons in the SON and PVN (Geffard et al., 1984). Both studies suggest (but have not proven) that the A11 cells within the periventricular dopaminergic system and/or the A13 and A14 cells in the anterior and dorsal hypothalamus are likely sources for this dopamine innervation.

The fact that dopamine terminals were found to make synaptic contacts with both cell bodies and dendrites of magnocellular neurons throughout the SON (Buijs et al., 1984; Decavel et al., 1987) suggests, but does not prove, that the DA innervation is present on both vasopressin and oxytocin cells. Final anatomical confirmation will require double label studies where the chemical composition of both pre and postsynaptic structures is clearly defined.

2.6.2.1.3. Histaminergic afferents

Both the SON and PVN contain histamine (Taylor et al., 1972; Brownstein et al., 1974) as well as a high density of \( H_1 \)-binding sites (Palacios et al., 1981a). The main source of
histamine in the rat brain is in the tuberomammillary nuclei of the posterior hypothalamus (Panula et al., 1984) and cells in this area project directly to the SON. Neurons in the tuberomammillary nuclei are labelled by retrograde transport following injections of tracer into the SON (Weiss et al., 1989), and fibers can be traced directly from tuberomammillary nuclei to the SON magnocellular cells.

Initial studies suggested that histamine stimulates the activity of SON neurons in vitro (Armstrong and Sladek, 1985) and in vivo (Haas et al., 1975). This has recently been confirmed by in vitro intracellular recordings in horizontal slices of rat hypothalamus where responsive neurons were labeled and immunocytochemically identified. It appears that both vasopressin- and oxytocin-stained SON neurons can be influenced by tuberomammillary histaminergic neurons. Vasopressinergic neurons are activated via $H_1$ receptors (Hatton and Yang, 1988) and oxytocinergic neurons are depressed via $H_2$ receptors (Yang and Hatton, 1989).

2.6.2.1.4. Angiotensinergic afferents

Angiotensin II (AII) is known to initiate physiological and behavioral responses which participate in the homeostatic maintenance of body water balance by acting at peripheral
sites and in the brain (Fitzsimons, 1980). The subfornical organ (SFO), a highly vascularized (Dellmann and Simpson, 1976) circumventricular organ of the brain, is an important receptor site for the dipsogenic action of AII in the induction of drinking behaviour (Simpson and Routtenberg, 1973; Simpson et al., 1978; Simpson, 1981; Lind and Johnson, 1982). Microinjections of AII into the SFO of the rat elicit specific and dose-dependent drinking (Simpson and Routtenberg, 1973; Simpson et al., 1978), while lesions of the SFO severely disrupt drinking in response to peripherally injected AII or to experimental hypovolemia (Hosutt et al., 1981; Simpson et al., 1978). These findings suggest that the SFO is a central receptor site for the dipsogenic effect of circulating AII.

Neurons in the SFO project to both the SON and PVN, as initially demonstrated by anatomical studies utilizing anterograde transport of tracer molecules (Lind et al., 1982, Miselis et al., 1979, Miselis 1981). This is a direct input to magnocellular neurons in the SON since lesions in the SFO cause axon terminal degeneration at this site (Renaud et al., 1983). This innervation from SFO appears to be part of a central circuitry that contains angiotensin immunoreactive neurons and may utilize this peptide as a neurotransmitter, a hypothesis based on the observations that the SFO has AII-immunoreactive neurons whose fibers project to the SON and PVN (Lind et al., 1984; Jhamandas et al., 1989a), and AII
receptors have been localized in the SON and PVN (Mendelsohn et al., 1984).

The electrical activity of both vasopressinergic and oxytocinergic SON neurons can be increased (a) by intravenous infusions of angiotensin (Ferguson and Renaud, 1986), an effect that is abolished by lesion of the SFO (Ferguson and Renaud, 1986) and (b) by stimulation of SFO (Sgro et al., 1984; Renaud et al., 1985), an effect that can be partially and reversibly antagonized by the angiotensin antagonist saralasin (Jhamandas et al., 1989a). These observations indicate that angiotensin II may act as a transmitter in specific central pathways to neurosecretory cells.

2.6.2.1.5. GABAergic afferents

The SON and PVN display high affinity binding for \([^3H]\)muscimol, a gamma-aminobutyric acid (GABA)-A receptor agonist (Palacios et al., 1981b) and both contain moderate levels of glutamic acid decarboxylase (GAD), the GABA synthesizing enzyme (see Tappaz et al., 1977). Immunocytochemical studies at the light and electron microscopic level also confirm that, jnocellular neurons, in particular those in the SON, receive a prominent GABA input (Tappaz et al., 1977; Van Den Pol, 1985; Theodosis et al.,
1986; Buijs et al., 1987; Jhamandas et al., 1989b). The source of this GABAergic input is not clear. But it has been suggested that the GABAergic interneurons are located in the perinuclear zone around SON and PVN (Theodosis et al., 1986). Areas where electrical stimulation produces depression of firing of magnocellular neurons (Poulain et al., 1980; Pittman et al., 1981; Hamamura et al., 1982; Thomson, 1982; Cirino and Renaud, 1985; Jhamandas and Renaud, 1986) include several sites that project to these perinuclear zones, e.g. the diagonal band (Swanson and Cowan, 1979; Jhamandas et al., 1989b), lateral septum (Swanson and Cowan, 1979; Oldfield and Silverman, 1985) and amygdala (Oldfield and Silverman, 1985).

2.6.2.1.6. Acetylcholinergic afferents

It has long been postulated that acetylcholine serves as a transmitter in pathways to the magnocellular system (Kühn, 1974; Sakai et al., 1974). Cholinergic facilitation of vasopressin release has been observed during both in vivo (Pickford, 1939; 1947; Barker et al., 1971a; Moss et al., 1971; Bhargava et al., 1972; Dreifuss and Kelly, 1972; Milton and Paterson, 1974: Bioulac et al., 1978) and in vitro (Sakai et al., 1974; Sladek and Joynt, 1979) investigations. The activity of neurosecretory neurons and the release of AVP from the neurohypophysis are increased by application of
acetylcholine and nicotinic agonists. These responses can be prevented by nicotinic antagonists (Barker et al., 1971a; 1971b; Dreifuss and Kelly, 1972; Sakai et al., 1974; Sladek and Knigge, 1977; Sladek and Joynt, 1979; Gregg, 1985; Mason et al., 1986). As for the release of OT, some reports indicate that acetylcholine has facilitatory effects both in vivo (Kühn, 1974; Clarke et al., 1978) and in vitro (Bridges et al., 1976) studies, although some report that it has no effect (Mason et al., 1986).

Meyer and Brownstein (1980) pointed out that surgical deafferentation of the SON had little effect on its content of choline acetyltransferase (CAT), a specific marker for cholinergic neurons, thus suggesting a local origin. The presence of a group of cholinergic (CAT) neurons located dorsolateral to the SON with processes entering the SON offers one possibility (Mason et al., 1983). In the SON, such an input may be more obvious on vasopressinergic neurons (Parent and Butcher, 1976; Sofroniew et al., 1982; Mason et al., 1983; Armstrong, 1985; Hatton and Mason, 1985). Both SON and PVN demonstrate binding for nicotinic receptor ligands, including [125I]α-bungarotoxin ([125I]α-BTX) (Segal et al., 1978; Meeker et al., 1986) and [3H]nicotine (Sharp et al., 1987).

2.6.2.1.7. Serotonergic afferents
Studies of the distribution of serotonergic fibers in the hypothalamus have shown that such input in the SON and PVN is quite sparse (Fuxe, 1965; Aghajanian et al., 1973; Kent and Sladek, 1978; Steinbusch, 1981), compared with that in the surrounding neuropil or parvocellular division of the PVN (Sawchenko et al., 1983). Projections to SON and PVN from the midbrain raphe nuclei (Conrad et al., 1974; Azmitia and Segal, 1978; Moore et al., 1978) appear to arise from the B7, B8 and B9 serotonergic cell groups (Sawchenko et al., 1983). It is interesting that more serotonergic fibers are present in the region where oxytocin-synthesizing cell somata are concentrated (Sawchenko et al., 1983). The role of serotonin in regulating vasopressin or oxytocin secretion remains controversial.

2.6.2.2. Other anatomically-defined afferents

2.6.2.2.1. Lateral septum and amygdala

In a combined electron microscopic HRP and immunocytochemical study, Oldfield et al. (1985) showed that there are direct synaptic contacts of lateral septum and amygdala efferents with vasopressin-containing dendrites emanating from the SON and/or PVN. Since the amygdala and the lateral septum also send projections to the perinuclear zones
around the SON and PVN (Zaborsky et al., 1975; Sawchenko and Swanson, 1983; Silverman and Oldfield, 1984; Tribollet et al., 1985), it is possible that this area contains the GABAergic cells that mediate inhibition exerted by the amygdala and lateral septum on SON and PVNmc cells.

2.6.2.2.2. Anteroventral third ventricle (AV3V) area

The anteroventral third ventricular (AV3V) region contains the organum vasculosum of the lamina terminalis (OVLT), median preoptic nucleus (MnPO, or nucleus medianus of the medial preoptic area), periventricular preoptic (PVPO) and medial preoptic areas (MPO). The OVLT, a circumventricular structure lacking a blood-brain barrier, is located within the rostral-most wall of the optic recess of the third ventricle and contains a densely reticulated net of fenestrated capillaries (Simpson, 1981). The MnPO encircles the medial third of the anterior commissure at the rostral border of the third ventricle. Both the OVLT and the MnPO receive inputs from the subfornical organ, another circumventricular structure (Miselis et al., 1979; Miselis, 1981). Neurons in all three of these areas (i.e. OVLT, MnPO and subfornical organ) project to the SON and PVN (Miselis 1981; Tribollet and Dreifuss, 1981, Silverman et al., 1981; Sawchenko and Swanson, 1982a; Renaud et al., 1983; Sawchenko et al., 1983; Tribollet
et al., 1985; Weiss et al., 1989, Wilkin et al., 1989). Stimulation in these areas also evokes changes in the excitability of neurosecretory neurons. For example, hyperosmotic media applied in the area of OVLT increases the firing of neurosecretory cells (Honda et al., 1987) as does electrical stimulation in the subfornical organ (Sgro et al., 1984; Ferguson et al., 1984; Gutman et al., 1986; Tanaka et al., 1986; Jhamandas et al., 1989a). The latter response may be mediated by angiotensin (Jhamandas et al., 1989a). In contrast, electrical stimulation of MnPO evokes mostly depressant responses from neurosecretory cells (Osaka et al., 1988; Leng et al., 1989; Nissen and Renaud 1989), mediated through GABA-A receptors (Nissen and Renaud, 1989). Neurons in the AV3V area and in the subfornical organ have been implicated in hydromineral balance (Miselis, 1981; Simpson, 1981; Johnson and Cunningham, 1987). Electrolytic lesions of the AV3V of the hypothalamus abolish osmotically stimulated AVP release both in vivo (Johnson et al., 1978; Gruber et al., 1986) and in vitro (Sladek and Johnson, 1983). The acute effects of AV3V lesions include abolition of the drinking responses to hypovolemia (Buggy and Johnson, 1977; Lind and Johnson, 1983) and adipsia to hyperosmotic stimuli (Johnson and Buggy, 1978).

Like AV3V lesions, lesions of the MnPo (Mangiapane et al., 1983; Gardiner and Stricker, 1985) or the OVLT (Thrasher
et al., 1982; Thrasher and Keil, 1987) also attenuate drinking and AVP secretion in response to AII and osmotic stimulation, but they do not cause adipsia. Therefore, both MnPO and OVLT have an important role in regulating drinking behaviour and in the release of hormones from the neurohypophysis. Since hormonal (vasopressin release) responses to haemorrhage are not affected by the OVLT lesion (Thrasher and Keil, 1987), it is suggested that the OVLT contains osmoreceptors involved in physiological responses to cellular dehydration.

2.6.2.2.3. The olfactory bulb

Mitral cells in the main olfactory bulb send projections to the ventrolateral portion of the SON, but not to the PVN (Smithson et al., 1989). Intracellular data from SON neurons recorded in vitro reveal that electrical stimulation of the lateral olfactory tract produces excitatory responses in a majority of AVP- and OT-immunoreactive neurons (Hatton and Yang, 1989). Potential transmitters remain to be identified.

2.7. Dopamine and Neurosecretion

2.7.1. Distribution in the infundibulum and hypophysis
The secretion of neurohypophysal hormones may be regulated not only at the level of the somata of the vasopressin- and oxytocin-synthesizing neurons but also at the level of the axon terminals in the pituitary. The posterior pituitary as well as the intermediate lobe are innervated by fibers which contain catecholamines (Björklund et al., 1967; 1970), serotonin (Björklund et al., 1967; Léránth et al., 1983), acetylcholine (Lederis and Livingston, 1970) and some other neurotransmitter substances (Holzbauer et al., 1984). Dopamine is the major catecholamine (Björklund et al., 1967; Saavedra et al., 1975; Holzbauer et al., 1978). These dopaminergic nerve fibers arise from the rostral part of the arcuate nucleus. The axons projecting to the intermediate lobe have been traced to the cell bodies in the most rostral zone of the arcuate nucleus whereas those innervating the posterior lobe originate in a small cell group immediately caudal to the former (Björklund et al., 1973a). These neurons form the so-called tuberohypophysial dopamine system, referred to as group A12 in the nomenclature of Fuxe et al. (1965). The short tuberohypophysial dopamine neurons appear to have specialized and orderly arranged projections to the intermediate and neural lobe of the pituitary (Björklund et al., 1968; 1973a; 1973b; Jonsson et al., 1972; Moore et al., 1980). In the neural lobe, these tuberohypophysial dopamine neurons which make intimate contact with the neurosecretory axons within the neurohypophysis terminate close to pericapillary spaces.
neurosecretory axons and pituicytes (Baumagarten et al., 1972). Recent evidence indicates that the dopamine neurons which innervate the neurohypophysis are actually independent from those innervating the intermediate lobe (Björklund et al., 1973a; Holzbauer and Racké, 1985).

Some perikarya of the group A12 neurons forming the so-called tuberoinfundibular dopamine system are also located in the arcuate nucleus. These neurons end on the hypophyseal portal vessels in the external layer of the median eminence (Moore et al., 1980; Moore and Demarest, 1982; Björklund et al., 1973a) and are independent from the dopamine neurons innervating the neural lobe and intermediate lobe (Moore and Demarest, 1982).

2.7.2. Dopamine receptor types

A dopamine receptor is defined as that receptor which is more sensitive to dopamine than to any other neurotransmitter (Grigoriadis and Seeman, 1984). There are at least two types of dopamine receptors in the brain. One type, termed D1 dopamine receptor, responds to micromolar concentrations of dopamine and is characterized by its ability to increase the synthesis of cyclic AMP (Kebabian et al., 1972; Kebabian and Calne, 1979; Brown and Makman, 1972; Iversen, 1975; Andersen
et al., 1990), by high sensitivity to specific D1 receptor agonists, such as SKF 38393 (Setler et al., 1978; Stoof and Kebabian, 1981; Watling and Dowling, 1981; Treiman and Greengard, 1985), and by insensitivity to benzamide drugs such as sulpiride (Grigoriadis and Seeman, 1984; Mason 1983). Another type, termed D2 dopamine receptor, responds to nanomolar concentrations of dopamine (Kebabian and Calne, 1979; Frey et al., 1982), is either inhibitory (De Camilli et al., 1979; Munemura et al., 1980; Stoof and Kebabian, 1981; Cote et al., 1982; Frey et al., 1982; Andersen et al., 1990) or not linked to cAMP formation (Kebabian and Calne, 1979; Andersen et al., 1990) and its action can be mimicked by D2 agonists such as quinpirole or LY 171555 (Plantjé et al., 1987; Itoh et al., 1985; Yang et al., 1989) or can be antagonized by (-)-sulpiride (Kebabian and Calne, 1979; Frey et al., 1982; Albert et al., 1990) and spiperone (Grigoriadis and Seeman, 1984; Albert et al., 1990). Recent studies indicate that there are possible subtypes of D1 and D2 receptors, as well as distinct D3 (Sokoloff et al., 1990) D4 (Van Tol et al., 1991) and D5 receptors (Sunahara et al., 1991).

2.7.3. DA receptors distribution and function: SON, PVN and neurohypophysis
Autoradiographic studies have shown high affinity binding for dopamine in both the area of magnocellular neurosecretory neurons (Leibowitz et al., 1982) and in the neurohypophysis (Ahn et al., 1979; Cronin and Weiner, 1979; Stefanini et al., 1980).

2.7.3.1. DA receptors on the magnocellular neurons

Mason (1983) has suggested that two classes of DA receptor exist in the SON, based on electrophysiological and pharmacological results. Mason attempted to differentiate dopamine receptors on the basis of their pharmacological sensitivity to DA antagonists. His results imply that the predominant receptor type for DA stimulation of putative oxytocin cells in the rat SON is of the D1 receptor class. However, investigations by Yang et al. (1989; 1990; 1991) using in vitro intracellular recordings reveal a slow onset depolarizing response from most of the SON neurons exposed to DA (1-100 μM) and to the D2 agonist quinpirole, but not to the D1 agonist SKF 38393. Moreover it appears that both AVP- and OT-secreting neurons respond similarly. Hence excitatory D2 receptors may be located on both cell types (see discussion).

2.7.3.2. DA receptors in the NIL
With respect to the function of D1 and D2 dopamine receptors in the isolated neurointermediate lobe (NIL) or neural lobe (NL), it has been proposed that D1 receptors facilitate (Racké et al., 1982a; 1982b; Treiman and Greengard, 1985) and D2 receptors inhibit the release of vasopressin (Racké et al., 1982a; Lightman et al., 1982; Treiman and Greengard, 1985). Dopamine has no effect on the vasopressin release evoked by ouabain (Pitzel and König, 1984) or by high potassium concentration (Racké et al., 1982a).

Dopamine itself is released from the rat basal hypothalamus (Plantjé et al., 1987), NIL (Plantjé et al., 1987; Holzbauer et al., 1983) or NL (Holzbauer et al., 1983) by electrical stimulation and high potassium (Sharman et al., 1982) in a calcium-dependent manner. The release of dopamine can be inhibited by D2 presynaptic dopamine autoreceptors (Racké et al., 1988; Plantjé et al., 1987).

2.7.3.3. Summary of dopamine influence on the activity of magnocellular neurons and on secretion

Both excitatory and inhibitory effects of dopamine have been reported. Tab. 1 lists effects of dopamine on the activity of AVP and OT secreting cells in hypothalamus and on the release of hypophysial hormones.
**Effect of dopamine**

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.c.v.</td>
<td>↓ AVP</td>
<td>Kimura et al., 1981</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>↑, then ↓ AVP</td>
<td>Forsling and Williams, 1984</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>↑ AVP</td>
<td>Iványi et al., 1986</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>↑ OT</td>
<td>Clarke et al., 1979b</td>
</tr>
<tr>
<td>iontophoresis</td>
<td>↑ activity of cells</td>
<td>Barker et al., 1971a</td>
</tr>
<tr>
<td>local injection</td>
<td>antidiuretic response</td>
<td>Urano and Kobayashi 1978</td>
</tr>
<tr>
<td>onto the SON</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intraventricular</td>
<td>↑ activity of cells</td>
<td>Moos and Richard, 1982</td>
</tr>
<tr>
<td>injection</td>
<td>↑ OT</td>
<td>Bridges et al., 1976</td>
</tr>
<tr>
<td>isolated hypothalamus</td>
<td>↑ AVP &amp; OT</td>
<td>Bridges et al., 1976</td>
</tr>
<tr>
<td>isolated hypothalamo-</td>
<td>↓ OT</td>
<td>Seybold et al., 1978</td>
</tr>
<tr>
<td>neurohypophysis</td>
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<td></td>
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<tr>
<td>hypothalamic slices</td>
<td>↑ activity of cells</td>
<td>Mason, 1983</td>
</tr>
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<td>hypothalamic explant</td>
<td>↑ activity of cells</td>
<td>Yang et al., 1989; 1990; 1991</td>
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<td>isolated neural lobe</td>
<td>↓ OT</td>
<td>Vizi and Volbekas, 1980</td>
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<td>isolated NIL</td>
<td>no effect on AVP &amp; OT</td>
<td>Hashimoto et al., 1988</td>
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<tr>
<td></td>
<td>↓ OT</td>
<td>Barnes and Dyball, 1982</td>
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</tbody>
</table>

Differences in species, doses, sites and times of administration, methods of measurement of hormones release, the nature of the preparation employed and initial activity of the magnocellular neurosecretory neurons may all contribute to the divergent findings.
2.8. Purpose of Experimental Study

On the premise that dopamine facilitates the firing of neurosecretory neurons, the present series of experiments were undertaken to evaluate the ability of dopamine to effect release of vasopressin from the neurohypophysis in an intraarterially perfused preparation of rat hypothalamus where it is possible to control variables that plague in-vivo studies. Experiments were designed to focus drug actions to the level of the somata of SON neurons and to rule out possible drug actions at other sites, notably in the neural lobe itself, by delivering drugs directly over the SON nuclei instead of into the perfusion medium and by determination of the effect of stalk damage on release.
3. MATERIALS AND METHODS

All experiments described in this thesis were performed in vitro with a perfused explant of rat hypothalamus (with pituitary attached) and utilized radioimmunoassay to measure AVP release from the neural lobe.

3.1 Hypothalamic Explant Chamber

The hypothalamic explant was incubated in a twin-chambered cylindrical bath (Fig. 3.1) whose outer chamber contained distilled water maintained at 35 °C thermostatically. The inner chamber (Fig. 3.1A) consisted of a well drilled into a thick perspex disc. The bottom of the chamber was filled to within 1-2 mm of the top with Sylgard elastomer into which pins were inserted to stabilize the hypothalamic explant.

3.2 Perfusion Medium
Perfusion medium resembled artificial cerebrospinal fluid (ACSF) and contained (in mM) NaCl(124), KCl(3), KH₂PO₄(1.25), MgCl₂·2H₂O(1.3), CaCl₂·2H₂O(1.2), NaHCO₃(25.9), and Glucose(10). The osmolality of the medium was tested by freezing-point osmometry (Advanced instruments micro-osmometer OSM) and adjusted when necessary to 298±2 mosmole/L by addition of distilled water. The medium was stored at room temperature under a 95% O₂ : 5% CO₂ atmosphere in reservoirs located about 1.5 meters above the bath and gravity-fed through PE-120 polyethylene tubing to the outer bath where it was heated to 35 °C. These PE tubing were led through two holes in the top of the outer chamber and connected to glass pipettes whose tip diameter of 150-200 μm permitted the medium to flow at a rate of 1.0-1.5 ml/min. These pipettes were fitted to micromanipulators to allow positioning in the stump of the internal carotid or anterior cerebral arteries. (Fig. 3.2)

In order to avoid possible loss of O₂ by diffusion from the medium while flowing from the reservoir to the bath, the PE-120 tubing carrying the medium from the reservoirs to the valves were surrounded by Tygon tubing through which a 95% O₂ : 5% CO₂ gas mixture was passed (Fig. 3.1). This gas was directed into the outer bath for humidification and then to the vicinity of the inner bath via holes drilled in the plate covering the outer bath so as to provide a warm, humidified, oxygenated environment for the explant. Fiberoptic
illuminators positioned above the bath provided heat-free transillumination of the explant.

3.3 Preparation for Hormone Release Studies

The experiments were carried out on male rats of the Long-Evans strain, weighing 175-220 g (Canadian Breeding Farm Laboratories). Following decapitation, the bones forming the roof of the skull were rapidly removed. The olfactory tubercles and optic nerves were severed. The dura surrounding the pituitary was carefully cut with fine scissors, together with the adjacent cranial nerves and blood vessels. The brain was then lifted forwards, and any remaining vessels were divided, allowing the brain to be removed with the pituitary still attached. The brain was gently lifted away from the skull and attached by its dorsal surface to the base of an inverted petri dish using cyanoacrylate glue. An explant of the basal forebrain measuring approximately 10x10x2 mm and including the hypothalamus, pituitary and parts of the temporal and frontal cortex, optic nerves and chiasm and mesencephalon was cut carefully with razor blades to preserve the entire vasculature of the Circle of Willis.

Following transfer to the inner bath, this brain explant was fixed in place with insect pins inserted into the Sylgard.
Micromanipulator-mounted perfusion pipettes with ACSF flowing were then inserted into the internal carotid arteries bilaterally and the pipette tips advanced to the bifurcation of the anterior and middle cerebral arteries. This caused the tissue to blanch rapidly. Generally, it took 7-10 minutes from decapitation to initiation of perfusion. Clotted blood was removed from arteries and veins with jeweler's forceps and the optic nerves were trimmed to the level of the optic chiasm, avoiding damage to surrounding tissues. The membranes overlying the SON were not disturbed except that the tips of micropipettes penetrated them during local injection. Damaged preparations were discarded.

In order to ease diffusion of secretory products of the neurohypophysis into medium, the anterior pituitary was removed using the fine scissors, leaving the neurointermediate lobe intact. Explants were incubated for 1 hour prior to testing.

After flowing through the vessels and out of their severed endings, the ACSF was allowed to collect in the well and cover the explant to a depth of approximately 1 mm. A suction pipette connected to a peristaltic pump (Fig. 3.2.) was positioned above the posterior pituitary and drew off ACSF at a speed of 1 ml/min to the fraction collector. During the 6-8 hour experiments, 1 or 2 minute fractions of effluent
medium were collected in 75x12 mm polystyrene test tubes which were put in ice during the collection and then transferred for storage at -20 °C until measured by radioimmunoassay.

3.4 Drug Applications

In order to test the efficacy of pharmacological agents (dopamine, noradrenaline, a D1 agonist: SKF 38393, and a D2 agonist: quinpirol hydrochloride (QNP)) to release neurohypophysial hormones, two methods of drug application were used. One was perfusion and the other was local injection onto the surface of each SON.

Perfusion: Two 30-gauge hypodermic needles were inserted into each perfusion line as shown in Fig. 3.1. and connected through PE 10 tubing to 1 ml syringes containing a concentrated solution of drug dissolved in control ACSF. Delivery of the drug was controlled by a syringe pump (Hamilton) in the speed of 0.6 ml/min.

Local injection onto the SON: Two micropipettes (tip diameter between 50 μm - 70 μm) (Fig. 3.2.) were connected via PE 20 tubing to 5 μl microsyringes containing a known concentration of drug dissolved in control ACSF. Delivery of the drug was also controlled by the same syringe pump in the
range of 0.5-6.0 µl/min. These microinjection pipettes were fitted to micromanipulators to allow positioning over the SON.

In some of the experiments, a D2 antagonist (spiperone hydrochloride or sulpiride hydrochloride) or an α₁-antagonist (prazosin hydrochloride) was applied in known concentration in the perfusion medium. Due to its poor solubility in aqueous solutions, prazosin hydrochloride was first dissolved in dimethyl sulfoxide (DMSO) and diluted with ACSF to the final concentration (Randle et al., 1986b). The final concentration of DMSO did not exceed 0.005%.

3.5 Radioimmunoassay of Vasopressin

Immunoreactive AVP determination was carried out using a specific radioimmunoassay (RIA) system developed with the assistance of Bert van de Heijning (Utrecht). Synthetic AVP (Peninsula) was used as standard and for preparation of tracer. The antisera were properly titred so as to bind approximately 30% of iodinated AVP. The final antibody dilutions used in the assay tube were 1:360,000 - 1:280,000. The antisera used were highly specific for AVP. The cross-reactivity of AVP (80 pg/ml) with OT (80 pg/ml) was less than 0.005%.
The diluent used in the AVP assay contained 28.7 mM Veronal (Barbituric acid) buffer, pH 8.0, 139 mM sodium chloride (NaCl), 10 mM disodium ethylenediaminetetraacetic acid (EDTA), 16 μg/ml L-cystine (Sigma Chemical Co.) and 1.25 mg/ml crystallized and lyophilized Human Albumin (Sigma Chemical Co.). Horse serum (GIBCO Laboratories, Life Technologies Inc.) was heat inactivated. The polyethylene glycol (Sigma Chemical Co.) used to precipitate antibody (Desbuquois and Aurbach, 1971) was prepared by dissolving 200 g in 1 litre distilled water. The tracer used for AVP was commercial (Amersham Canada Limited).

Albumin was used to avoid adsorption of AVP to the tubes (Oyama et al., 1971). Only crystallized albumin was used since most of the less purified preparations have been found to interfere significantly in the assay (Robertson et al., 1973). The horse serum served as carrier (Salacinski et al., 1981) to avoid adsorption of antibody. EDTA and cystine were added to prevent degradation of AVP during incubation (Oyama et al., 1971).

All samples were incubated in 75x12 mm plastic test and centrifuge tubes (SARSTEDT, Canada Inc.).

Briefly, after preparing serial double dilutions (range 1:20 to 1:5,120) of standard AVP (2560 pg/100 µl), 50 µl
triplicate aliquots were incubated with 50 µl W1E (AVP antibody, 1:360,000-1:280,000, according to the antibody dilution curve) for 48 hours at 4°C. Each tube then received 10 µl diluted solution of [125I]-AVP so as to attain approximately 7,500 - 8,500 cpm in 30 sec., and incubated for 24 hours at 4°C. After addition of 50 µl of horse serum and 500 µl of 20% polyethylene glycol, each tube was centrifuged at 3,000 rpm at 4°C for 20 minutes, the supernatant aspirated and the tubes placed in a gamma-counter for 3 minutes counting time each. A standard curve construction is illustrated in Fig. 3.3. Fig. 3.4. illustrates an example of an antibody dilution curve.
Fig. 3.1.
Fig. 3.1. Diagram of the perfusion apparatus. Figure taken from Randle (1985).

(A): The perfusion (inner) chamber consists of a well drilled into a thick perspex disc. The bottom of the chamber is filled with Sylgard elastomer into which pins are inserted to stabilize the explant.

(B): The entire perfusion system: 1) infusion lines; 2) thermostatic temperature regulator; 3) fiberoptic light source; 4) micromanipulator and perfusion pipette; 5) perfusion chamber; 6) water jacket; 7) pressurized 95% O₂ with CO₂ source; 8) ACSF reservoir; 9) jacketed perfusion line; 10) pressurized outflow of the perfusion line jackets; 11) selected ACSF output perfusion line; 12) solenoid valve assembly; 13) waste drain.
Fig. 3.2.
Fig. 3.2. Experimental set-up for collection of perfusion medium for hormone assay. Figure from Randle (1985). Both internal arteries are cannulated with perfusion pipettes. This figure also illustrates two microinjection pipettes positioned directly over each supraoptic nucleus, and used for local drug administration. Medium is withdrawn by a suction pipette positioned caudal to the neurointermediate lobe (anterior pituitary removed), connected to PE 100 polystyrene tubing and collected in test tubes. AVP level is determined by RIA.
Fig. 3.3.
Fig. 3.3. AVP standard curve.

% of tracer bound is plotted against the natural log concentrations of standards (0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 pg) which contains $^{125}$I-AVP and synthetic AVP. The results of samples are expressed in terms of these standard curves, hence by the % of tracer bound. The minimal detectable amount or sensitivity of the AVP assay is 0.5 to 1.0 pg (10 to 20 pg/ml).
Fig. 3.4.
Fig. 3.4. AVP antibody (W1E) dilution curve.

% of tracer bound is plotted by concentrations of AVP antibody (W1E) in the range of 1:20,000 to 1:640,000. The final antibody dilution that gives 30% binding to iodinated AVP is then calculated (in this example, 1:360,000).
4. RESULTS

4.1. Base Line Release of AVP

During 1 hour period of incubation of the hypothalamic explant, 1 minute fractions of effluent medium were collected at intervals of 5 minutes. Spontaneous release of AVP was high initially, gradually declined over the initial 30 minutes and usually stabilized in the following half hour (see Fig. 4.1.).

All release data reported indicate release in excess of the base line level.

4.2. Definition of "Control"

From the outset it became obvious that the amount of hormone released differed markedly between explants. The reasons for this are not entirely clear but are likely to include damage due to stretch that axons of the neurohypophysial tract sustain during removal of the preparation from the cranial vault. It was necessary to
develop a test for use as a "control" that would on the one hand serve to test the viability and magnitude of response of each explant, and on the other hand act as a reference point for the hormonal response to dopamine and its agonists. On the basis of previous experience with noradrenaline and its consistent ability to release AVP (Randle et al., 1986a), it was decided to use the amount of AVP released by each explant in response to a 150 second exposure to 60 µM noradrenaline as the "control". Of the 65 explants tested, only 31 (48%) released AVP in response to a 60 µM noradrenaline infusion and therefore were used for the data base. In this group, the total noradrenaline-evoked response (measured as total pg of AVP released over baseline in the 20 minutes interval following onset of the infusion) served as "control" for the dopamine-release data.

The results below are divided in two groups: the first 15 experiments utilized drugs applied in the perfusion line, the second 22 experiments, utilized local drug application by superfusion over each SON area.

In local drug superfusion, drug solutions were applied directly over the ventral surface of the SON by using the microinjection pipettes. In control experiments, up to 1 µl of ACSF (250 nl, 500 nl and 1 µl) applied over each SON failed to evoke any significant hormone release.
4.3. Effect of Noradrenaline on AVP Release

As stated above, on the basis of the present observations and of earlier studies (Randle et al., 1986a), one of the agents which most consistently evoked hormone release in this preparation is noradrenaline.

The response (AVP release) of these explants to the application of noradrenaline is typically delayed, and lasts for several minutes. This is seen in both perfusion (Fig. 4.2.) or superfusion (Fig. 4.3.) experiments where the amount of AVP in each fraction of effluent medium rises to a peak by the third or forth fraction, and then gradually declines. This response depends on the integrity of the hypothalamic-pituitary axis since the release of AVP is greatly decreased by cutting the pituitary stalk in both perfusion (Fig. 4.2.) and superfusion (Fig. 4.3.) experiments.

The "total" release of AVP in response to noradrenaline (60μM used in perfusion; 150μM used in superfusion) was obtained by adding the total amount of AVP in all fractions exceeding the mean baseline level in the 8 fractions (16 minutes, in perfusion) or 10 fractions (10 minutes, in superfusion) that were collected following drug administration (Fig. 4.4.). The large standard deviations reflect marked
variability in AVP release between experiments. The largest response in perfusion experiments was 2898 pg, and 948 pg in superfusion experiments.

4.4. Effect of an $\alpha_1$ Adrenergic Receptor Antagonist

In previous electrophysiological studies, the excitation of SON neurons by noradrenaline was reversibly antagonized by the selective $\alpha_1$ antagonist prazosin (Randle et al., 1986b) as well as by the non-selective alpha adrenergic antagonist phentolamine (Randle et al., 1986a). Hence it was considered relevant to determine that the release of AVP by noradrenaline did indeed occur through $\alpha_1$ receptors.

In perfusion experiments, 50 nM prazosin greatly reduced the effect of 60 $\mu$M noradrenaline. In one of the better experiments, the AVP response to noradrenaline decreased from 2799.0 pg (control) to 561.7 pg (See Fig. 4.5.). In superfusion experiments, the same (50 nM, n=2) or even higher (200 nM, n=1) concentrations of prazosin did not have such effect. Only when the prazosin concentration was increased to 1 $\mu$M (n=4) or 5 $\mu$M (n=1), was there an apparent reduction of the AVP response (Fig. 4.6.).
In these experiments with prazosin, recovery of the response to noradrenaline was only partial after 1-2 hours, by which time the viability of the explant was questionable. Hence there are no post prazosin recovery data available.

4.5. Influence of Dopamine on AVP Release

4.5.1. General observations

Release of AVP could be evoked by dopamine at 600 μM and 1.5 mM concentrations in perfusion and superfusion experiments, respectively. As with noradrenaline, the response of AVP release to dopamine also varied between explants. Fig. 4.7 displays the actual data from a perfusion experiment in which both dopamine and noradrenaline increased AVP release. Despite the use of a 10 fold greater concentration of dopamine compared with noradrenaline, the AVP response to dopamine was consistently lower than that to noradrenaline. In this instance application of a D2 dopamine receptor blocker (0.1 μM sulpiride) had no effect on the dopamine-evoked release of AVP.

4.5.2. Perfusion experiments
The AVP response to 60 μM (n=6) and 600 μM (n=12) dopamine was evaluated. As shown as Fig. 4.8., in one particular experiment the total amount of AVP released by 600 μM dopamine was 300.7 pg, whereas AVP released by 60 μM dopamine was only 25 pg. However when all data were evaluated, there was no significant difference (p>0.05) between these two groups.

4.5.3. Superfusion experiments

Dopamine applied by superfusion also had facilitory effects on the release of AVP. Three different concentrations of dopamine (15 μM (n=3), 150 μM (n=2) and 1.5 mM (n=8)) were applied. Although individual experiments demonstrated a dopamine-stimulated AVP release in a dose dependent manner (Fig. 4.9.), there was no significant difference (p>0.05) in AVP released by these three different concentrations when all data were considered.

4.5.4. Integrity of the hypothalamic-pituitary axis

The response (AVP release) of these explants to the application of dopamine was typically delayed, and lasted for several minutes. As illustrated in Fig. 4.10. (perfusion) and
Fig. 4.11. (superfusion), the release of AVP in each fraction of effluent medium rose to a peak by the forth fraction, and then gradually declined. As illustrated in Figs. 4.10. and 4.11. it is very important to maintain the integrity of the hypothalamic-pituitary axis as the release of AVP is greatly decreased by cutting the pituitary stalk in both perfusion and superfusion experiments.

4.6. Dopamine Receptor Agonists

The following series of experiments were conducted to provide preliminary data on the effects of dopamine receptor agonists and antagonists. No attempt was made to evaluate dose -response relationships.

4.6.1. Perfusion experiments

The D1 dopamine receptor agonist SKF 38393 applied at 0.5 mM increased AVP release (n=5). The D2 dopamine receptor agonist QNP applied at 0.4 mM also increased AVP release (n=4). Figs. 4.12. and 4.13. are examples of release of AVP induced by either QNP, SKF 38393 or dopamine.
4.6.2. Superfusion experiments

The D1 dopamine receptor agonist SKF 38393, at the 0.5 mM concentration, stimulated AVP release (n=5). This effect was comparable to that after dopamine 1.5 mM (n=8). The D2 dopamine receptor agonist QNP, at the 0.5 mM concentration, also stimulated the release of AVP (n=5). In one study, the effect of the D1 agonist SKF 38393 and the D2 agonist QNP at the same concentration (0.5 mM) was comparable to the response to dopamine at 1.5 mM (Fig. 4.13.).

4.7. Effect of D2 Dopamine Receptor Antagonists

4.7.1. D2 antagonists vs dopamine

4.7.1.1. Superfusion experiments

Two concentrations of the D2 dopamine receptor antagonist spiperone, (10 μM and 50 μM) were used. 10 μM spiperone blocked the AVP response to dopamine by 95.7% whereas 50 μM, spiperone was 100% effective. Fig. 4.14. shows the result from one explant demonstrating selectivity of the spiperone block. The dopamine action was depressed by 99% (from 27.7 to 0.3 pg) by 10 μM spiperone, while the AVP release evoked by noradrenaline was little changed.
Fig. 4.1.
Fig. 4.1. Base line of AVP release.

During a 1 hour period of incubation of the explant following installation in the chamber, 1 minute fractions of effluent medium were collected at intervals of 5 minutes. Note that levels of AVP were quite high at the beginning, but decreased within 30 minutes and remained stable thereafter.
Fig. 4.2.

Perfusion

stalk section

AVP (pg/ml)

80μM NA

80μM NA
Fig. 4.2. Data from a perfusion experiment where 60 μM noradrenaline (0.3 ml) was applied in each perfusion line (open box). Note that the amount of AVP in each fraction of effluent medium rises to a peak (89.1 pg/ml) by the forth fraction (each fraction = 2 minutes) and then declines gradually. On the right, the release of AVP in response to 60 μM noradrenaline is greatly reduced after sectioning the pituitary stalk.
Superfusion

Fig. 4.3.
Fig. 4.3. Data from a superfusion experiment illustrate the release of AVP in response to 150 μM noradrenaline (250 nl, each side). AVP levels peak (99.4 pg/ml) by the third fraction (each fraction = 1 minute), then gradually decline. The release of AVP is markedly decreased after sectioning the pituitary stalk.
Noradrenaline

Fig. 4.4.
Fig. 4.4. In 15 explants in which 60 μM noradrenaline (0.3 ml) was applied in each perfusion line, the total AVP release per explant was 765.3 ± 750.6 pg (Mean ± S.D.). In superfusion experiments, a solution of 150 μM noradrenaline was applied in either 500 nl or 250 nl amounts over each SON. AVP release per explant was 356.4 ± 142.1 pg (n=3) or 267.7 ± 289.5 pg (n=14) respectively. There was no significant difference between responses to 500 nl or 250 nl.
Fig. 4.5.
Fig. 4.5. Data from an experiment in which the response to 60 μM noradrenaline applied by perfusion was partially blocked by the addition of 50 nM prazosin to the perfusion media. The total AVP released decreased from 2799.0 pg (control) to 561.7 pg in the presence of prazosin.
Fig. 4.6.

% of control response to 150uM NA

Prazosin concentration (uM)
Fig. 4.6. Summary of observations from superfusion experiments where bath applied prazosin appears to have a dose response effect in reducing the response to noradrenaline (NA), but only at high antagonist concentrations.
Fig. 4.7.
Fig. 4.7. In a perfusion experiment each column reflects the (above baseline) AVP levels in successive fractions collected after a 150 sec. administration of 0.6 mM dopamine (DA, with or without bath applied 0.1 µM sulpiride) and 60 µM noradrenaline (NA). Drugs were applied at 1 hour intervals. Both dopamine and noradrenaline increased AVP release. Note that the AVP response to noradrenaline is greater than to dopamine, whereas sulpiride has no influence on the response to dopamine at this concentration.
Fig. 4.8.

Dopamine concentration

AVP (pg)

0 50 100 150 200 250 300 350 400

60uM 600uM
Fig. 4.8. In a perfusion experiment, two different dopamine concentrations were used: 60 μM and 600 μM. Dopamine 600 μM evoked 300.7 pg of AVP, whereas dopamine 60 μM only stimulated 25.0 pg AVP release.
Fig. 4.9.

Dopamine concentration

150µM  1.5mM

AVP (pg)
Fig. 4.9. In a superfusion experiment, two concentrations (150 μM and 1.5 mM) of dopamine were tested. Dopamine stimulated AVP release in a dose-dependent manner. It increased higher AVP release (89.9 pg) at 1.5 mM concentration than that (12.7 pg) at 150 μM concentration.
Perfusion

Fig. 4.10.
Fig. 4.10. Data from a perfusion experiment where 0.6 mM dopamine (0.3 ml) was applied in each perfusion line (open box). Note that the amount of AVP in each fraction of effluent medium rises to a peak (39.9 pg/ml) by the forth fraction (each fraction = 2 minutes) and then declines gradually. On the right, the release of AVP in response to 0.6 mM dopamine is greatly reduced after sectioning the pituitary stalk.
Fig. 4.11.

Superfusion

stalk section

AVP (pg/ml)

1.5 mM DA

1.5 mM DA
Fig. 4.11. Data from a superfusion experiment illustrate the release of AVP in response to 1.5 mM dopamine (250 nl, each sidω). AVP levels peak (85.7 pg/ml) by the forth fraction (each fraction = 1 minute), then gradually decline. The release of AVP is markedly decreased after sectioning the pituitary stalk.
Fig. 4.12.
Fig. 4.12. 0.4 mM QNP had similar effect as 0.6 mM dopamine on AVP release in perfusion experiments. QNP and dopamine increased AVP release at levels of 54.3 pg and 104.9 pg, respectively.
Fig. 4.13.
Fig. 4.13. In one superfusion experiment that compared 0.5 mM QNP (D2 agonist), 0.5 SKF 38393 (D1 agonist) effect with that of 1.5 mM dopamine, all are seen to increase AVP release. 0.5 mM QNP, 0.5 mM SKF 38393 and 1.5 mM dopamine increased AVP release at levels of 296.7 pg, 336.3 pg and 406.0 pg, respectively.
Fig. 4.14.
Fig. 4.14. Data from one experiment to illustrate apparent selectivity of the action of spiperone. In a superfusion experiment, the effect of 1.5 mM dopamine on AVP release was blocked by 10 μM spiperone while the response to superfused noradrenaline was not affected.
5. DISCUSSION

The experiments described here provide data on the actions of two catecholamines (i.e. noradrenalin and dopamine) that participate in central mechanisms controlling the release of AVP. The strategy of utilizing hormonal release from the neurohypophysis of explants of rat hypothalamus represents an effort to focus attention on the final common neurosecretory pathway, i.e. the supraoptic-neurohypophysial pathway.

As reported earlier (Section 3.3), the response of the system to be tested depends on its integrity, which is a factor that is difficult to control given that the preparation must be removed intact from the cranium. A technical problem is fragility of the experimental preparation, presumably the cause of the marked variation in response to the same test dose from one preparation to the next. This variability may reflect the number of intact neurons and axons in the supraoptic-hypophysial pathway. Unfortunately the success or failure of an experiment can not be predicted until the RIA is performed. Since 40% of the experiments were not successful,
the number of trials in several of the protocols remained low, thus precluding the establishment of a proper dose-response testing for agonist-antagonists as is usually done in most pharmacological studies. Data from preparations in which the AVP release was not reproducible in successive drug trials were excluded from the analysis.

Given that the preparation is satisfactory, the next premise involves locus of drug action. Certain drugs (e.g. dopamine) may act at both the level of the cell soma, and the axon terminals, possibly with different results, thereby requiring alteration of experimental design. If the assay system is sufficiently sensitive and reliable to determine if hormone release has in fact taken place, the site of drug action at the level of the cell soma can be verified by examination of the response of this explant to acute stalk section. Indeed, this procedure drastically reduced the AVP response to dopamine or noradrenaline.

5.1. α-Adrenergic Stimulation of AVP Release

The present observations confirm that noradrenaline in concentrations of $10^{-5}$ to $10^{-4}$ M can stimulate neurohypophysial hormone release from a hypothalamic explant preparation (Randle et al., 1986a). The new information contributed by the
present study is that noradrenaline is also effective when given by local superfusion over the SON area of the hypothalamic explant. The observation that prazosin, an \( \alpha_1 \) receptor antagonist, blocks the stimulatory effect of noradrenaline confirms that the facilitory effect on release of hypophysial hormones may be mediated via an \( \alpha_1 \) receptor. This is consistent with previous electrophysiological (Randle et al., 1984; Armstrong et al., 1986; Inenaga et al., 1986) and hormone release data (Randle et al., 1986a; Armstrong et al., 1986; Willoughby et al., 1987). The results also agree with previous observations by Milton and Paterson (1974) who found that local injection of noradrenaline into the SON of cat increased blood levels of AVP. In the present experiments, effective concentrations of prazosin appear to be high for reasons that are not clear. Tests with other more soluble adrenergic antagonists might have yielded clearer results.

5.2. Dopaminergic Stimulation of AVP Release

Based on literature reports, dopamine has excitatory (Moss et al., 1972a; Bridges et al., 1976; Urano and Kobayashi, 1978; Clarke et al., 1979b; Moos and Richard, 1982; Mason, 1983; Iványi et al., 1986), inhibitory (Seybold et al., 1978; Kimura et al., 1981; Barnes and Dyball, 1982) or no consistent effect (Bhargava et al., 1972; Hoffmann et al.,
1977; Olsson, 1970) on the release of vasopressin and/or oxytocin. In the perfusion experiments reported here, dopamine in concentrations of $10^{-5}$-$10^{-3}$ M range stimulate AVP release.

5.2.1. D2 receptors facilitate AVP release

In order to establish whether the site of action of dopamine is in the hypothalamus, notably in the SON area, agonists were injected locally onto the SON. Dopamine still stimulated AVP release from the neurohypophysis when applied by this route. QNP, a specific D2 dopamine agonist mimicked the dopamine effect when applied locally onto the SON. It is of interest that the D2 dopamine antagonist spiperone, applied in the ACSF, reduced the dopamine action (Fig. 4.14.) although the concentrations used receptor specificity. These observations are consistent with recent electrophysiological data (Yang et al., 1989, 1990, 1991) showing that SON cells depolarize in the presence of dopamine and QNP.

5.2.2. D1 receptors also influence AVP release

In contrast with the observations of Yang et al. (1991), the present experiments indicate that SKF 38393, a specific D1 agonist, can also stimulate AVP release, a finding that agrees
with the earlier electrophysiological observations of Mason (1983). Unfortunately, D1 antagonists were not used in the study due to limited time and further work will be needed to confirm the role of D1 dopamine receptor in stimulation of AVP release.

5.2.3. The effect of dopamine is not mediated through an α-adrenergic receptor mechanism

In perfusion experiments, the stimulatory effect of dopamine on AVP release was reduced in the presence of prazosin, an α₁ receptor blocker. Since dopamine may act at adrenergic receptor sites (Day and Roach, 1976; Goldberg, 1972; Henning and Rubenson, 1970), it was considered that dopamine was acting on an α₁-adrenergic receptor to increase hypophysial hormone release. Using localized dopamine injections, over the SON area, it was evident that the adrenergic blocker was more effective against noradrenaline than against dopamine. For example, prazosin decreased dopamine action from 100% of control to 77.8% of control and decreased NA effect from 100% to 25.8% of control, indicating a possibly greater action on adrenergic receptors.
5.2.4. Importance of an intact hypophyseal stalk for hormone release

In both perfusion and local injection studies, the integrity of the pituitary was critical for a response, i.e. the release of neurohypophysial hormones. After cutting the stalk, but keeping the pituitary in the chamber, minimal hormone release was observed in response to either noradrenaline and dopamine stimulations. Thus during the preparation of the explant, it was considered very important to avoid any kind of damage of the stalk. This may explain why only 31 of the 65 preparations tested released AVP after application of noradrenaline. These preparations therefore formed the basis of the data presented.

5.3. Perfusion vs. Local Drug Administration

A dense capillary network within the SON (Ambach and Palkovits, 1979) is a likely explanation of why intravascular perfusion of ACSF can maintain hypothalamic explant viability for up to 12-15 hours. During the initial incubation period, explants do undergo a period of swelling and edema which may disrupt any existing blood-brain barrier. Hence drugs administered into the perfusion media can be expected to have almost direct access to the extracellular space around
neurons. One disadvantage of this preparation is that drugs applied by this intravascular route may act anywhere in addition to the SON, including the neural lobe of the pituitary. Given that the latter also contains receptors which may exert actions functionally different from those of receptors located on cell somata, this may result in data that conflict with results arising from the application of drugs in simpler systems e.g. the isolated neural lobe.

The advantage of localized drug injection is the restriction of the site of action to the area of the SON, therefore somewhat analogous to the study of Milton and Paterson (1974) who used intracerebral injection into the SON itself. The superfusion method used here is advantageous since it is less likely to cause tissue damages; the disadvantage relates to difficulty for the drug to penetrate into the tissue. In any event, since the injected volume never exceeded 1 μl, the spread can be assumed to be confined to a sphere approximately 1 mm in diameter (Myers, 1966). This was confirmed by dye test in the present study. Even with drug application limited to the SON region, drugs act both directly on SON neurons as well as on cells in the vicinity of the nucleus (Milton and Paterson, 1974). Therefore it is necessary to view the data obtained in the present study in combination with data from cellular electrophysiological studies which can determine whether SON neurons do indeed have functional...
receptors for the drug utilized. In the case of dopamine, Yang et al., (1991) have defined a D2 mediated depolarization of SON neurons, thus supporting the inference that results obtained here with dopamine and D2 agonists are mediated by functional receptors on SON neurons.
The present study evaluated the ability for noradrenaline and dopamine to evoke AVP release from perfused explants of rat hypothalamus. Noradrenaline (in concentrations of 60 μM in perfusion experiments and 150 μM in superfusion experiments) stimulated AVP release from the neural lobe. This effect was blocked by an α₁-adrenergic receptor antagonist, prazosin (50 nM in perfusion experiments; 1 μM in superfusion experiments). Dopamine at 0.6 mM concentration in perfusion experiments or at 1.5 mM concentration in superfusion experiments also stimulated hormone release. The effect of dopamine could be mimicked by both a D1 dopamine receptor agonist (SKF 38393) and a D2 dopamine receptor agonist (QNP) at 0.5 mM concentration. In selected experiments a D2 dopamine receptor antagonist, spiperone (10 μM) blocked dopamine effect. It is proposed that both noradrenaline and dopamine have facilitatory influence on AVP release in this preparation and the observed responses to dopamine appear to be mediated by both D1 and D2 dopamine receptors.
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