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OPTIMIZING RELEASE FROM PEPTIDE HORMONE SECRETORY NERVE TERMINALS

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Summary

Secretion of the peptide neurohormones oxytocin and vasopressin from terminals of magnocellular neurones in the mammalian neurohypophysis is elicited by conduction of depolarizing action potentials into terminal membranes, inducing opening of voltage-sensitive Ca^{2+} channels, entry of Ca^{2+} from the extracellular space and a rise in cytoplasmic Ca^{2+} concentration. The amount of peptide released per action potential is not immutable. In particular, the patterns in which action potentials are generated at the cell somata of the two types of neurone each appear exquisitely suited to optimize the release process at the terminal by utilizing a frequency-facilitation mechanism and by minimizing a mechanism of fatigue in the release process. The different properties of oxytocin and vasopressin neurones are of important physiological significance. The secretory terminals are also a site of receptor-mediated influences of neuromodulators which can greatly alter release efficiency. The mechanisms underlying facilitation and fatigue are not clearly understood. The evidence suggests that processes both prior to depolarization of the terminals (propagation and form of the action potentials) and directly at the terminals (frequency/pattern-dependent Ca^{2+} entry and channel openings) are involved. Transient activity-related increases in extracellular K^+ concentration may be involved at both sites. Two types of neuromodulation have been partly characterized. κ-Opioid receptors in secretory terminal membranes directly modulate depolarization-evoked peptide release probably *via* interactions with Ca^{2+} channels. β-Adrenergic receptors localized on neurohypophyseal astroglial cells mediate more subtle effects of noradrenaline. In the more chronic situation the neurohypophyseal astroglia alter their morphological relationships with neurosecretory elements and the basal lamina at release sites, changes which may also serve to optimize the secretory process.

Introduction

As with all neurones, the peptide-secretory neurone exerts control of release of its products at distant axon terminals by generation of somatodendritic action potentials. These are conducted along axons into terminal membranes where depolarization results in opening of voltage-sensitive Ca^{2+} channels, entry of

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extracellular Ca^{2+} and a rise in the intraterminal cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$). Information sensed at the cell body and dendrites in any number of forms, e.g. ephaptic, electrotonic, ionic, synaptic, parasynaptic or endocrine, can be translated into altered electrical activity in addition to altered genetic expression, synthesis and packaging of secretory product. Without necessitating altered synthetic activity, the amount of peptide released at terminals per action potential can be acutely controlled and is not immutable. Optimization and modulation of the electrical message can be classified into two categories. First, activity-dependent modulation occurs by generation of action potentials in patterns which greatly alter the efficiency of the release process at the terminals. Second, receptor-mediated modulation by other neuroactive molecules occurs at the secretory terminal region. In this article I shall attempt to describe these processes, what we know of their mechanisms and also the physiological importance of optimization and modulation of the release process.

The magnocellular neurones in the rat, secreting the nonapeptide hormones oxytocin (OXT) and arginine-vasopressin (AVP), are the best-studied mammalian peptidergic neurones and serve as the model in all the studies I shall describe. The cell bodies of these neurones are located principally in the supraoptic and paraventricular nuclei of the hypothalamus and send single axons which run together in the infundibular stalk. In the highly vascularized neurohypophysis each axon branches or buds to yield many secretory terminals abutting the pericapillary space. Since these neurones are required to maintain systemically active concentrations of the two hormones, the neurohypophysis represents the greatest concentration of neuropeptides found in the brain. This has allowed ready measurement of their secretory output. The anatomically discrete terminal region has also enabled ready study of the behaviour of the terminals isolated from their cell bodies *in vitro*.

Activity-dependent modulation

Facilitation and fatigue

The patterns of electrical activity generated at the cell bodies of OXT and AVP neurones are so characteristically different as to be virtually diagnostic in a number of situations (see Poulain & Wakerley, 1982). OXT neurones fire action potentials in a relatively continuous manner at low to moderate frequencies (1–10 Hz) except during parturition and lactation when discrete higher-frequency bursts (10–80 Hz for 1–4 s) are superimposed (Wakerley & Lincoln, 1973; Summerlee, 1981). AVP neurones, particularly when activated during, for instance, dehydration, exhibit a phasic pattern of activity with trains of moderate frequency discharge (3–15 Hz) lasting 4–100 s (mode 5–25 s) separated by similar periods of electrical quiescence. Each train is often initiated with a few seconds at a higher firing rate (up to 20–30 Hz) which then plateaus to a lower level (Poulain & Wakerley, 1982).

By applying these patterns of electrical impulses through stimulating electrodes

to the isolated neurohypophysis it is possible to demonstrate that each pattern is exquisitely suited to optimize the release process in the two types of secretory terminal. Optimization involves utilizing a property of frequency facilitation and minimizing a process of fatigue in the release mechanism.

Application of a given number of impulses at increasing frequencies results in a facilitation of the amounts of peptide released per impulse (Fig. 1). The two types of terminal, however, show different ranges of frequency-dependent secretion. AVP secretion is maximized around 10–15 Hz, no further increases and even a decrease in release per impulse being evident at higher frequencies (Fig. 1; see Dutton & Dyball, 1979; Knepel & Meyer, 1983; Bondy, Gainer & Russell, 1987). OXT secretion, however, shows increasing release per impulse up to a much higher frequency range.

During sustained continuous application of moderate-frequency regular impulses AVP release per impulse begins to decline rapidly whilst that of OXT is relatively well maintained and release per pulse declines over a much longer time course (Fig. 2; see Bicknell *et al.* 1984). This phenomenon of fatigue in the secretory process is reversible, and short 'silent' periods without impulses result in enhanced initial rates of secretion when stimulation is resumed (Bicknell *et al.* 1984). Significant recovery from fatigue is seen when the duration of silent periods is in the range 10–20 s, although further recovery is seen with longer silent periods (Bicknell *et al.* 1984; Cazalis, Dayanithi & Nordmann, 1985).

Physiological significance of facilitation and fatigue

The very different patterns of activity generated by OXT and AVP neurones are

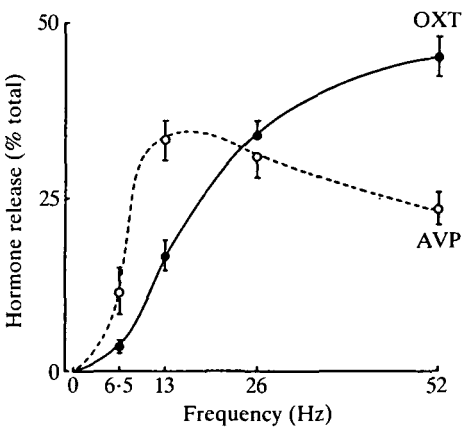

Fig. 1. Frequency facilitation of peptide release from oxytocin (OXT) and vasopressin (AVP) terminals stimulated electrically *in vitro*. Neurohypophyses received 156 pulses delivered at each of the four frequencies indicated in a balanced order of presentation. Peptide release evoked was determined by radioimmunoassay and normalized by expression as a percentage of the total release evoked by the four stimulations in each experiment. Note that frequency facilitation of release occurs over a lower range for AVP than for OXT terminals. $N = 8$; values are means ± S.E.

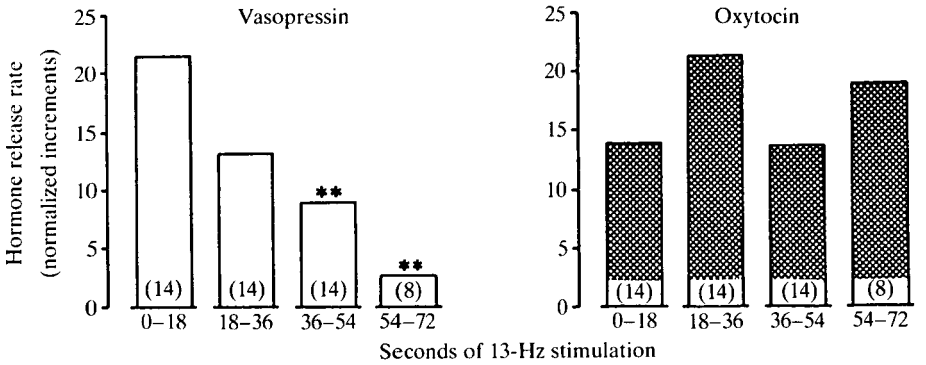

Fig. 2. Secretory fatigue in release of neurohypophyseal peptides. Peptide release evoked during consecutive 18-s periods of continuous electrical stimulation at 13 Hz. Stimulus durations were presented in a balanced order and evoked release is normalized to the total released by 18-, 36- and 54-s stimulations in each experiment. Note that the amount of vasopressin released declined rapidly with each 18 s of continuous stimulation but the output of oxytocin was relatively steady. ** $P < 0{\cdot}01$ *vs* 0–18 s release rate. *N* values are given in parentheses. Data are redrawn from Bicknell *et al.* (1984).

remarkably suited to differing properties of action potential–secretion coupling at the axon terminals. To exert its biological effects of contraction of mammary myoepithelia for milk ejection and of uterine muscle during parturition, OXT must be delivered in a bolus at an effective concentration (Dreifuss, Tribollet & Mühletaler, 1981). At milk ejection this is known to be achieved by the synchronous high-frequency discharge of OXT neurones resulting in a large, facilitated release from a relatively small number of action potentials. In contrast, to exert its diuretic action at the kidney during sustained dehydration, AVP must be released at a maintained elevated rate. This is achieved not by synchronous electrical discharge but by each neurone adopting a characteristic phasic pattern of discharge which facilitates release per impulse during the initial stages of a burst (Cazalis *et al.* 1985) and, by the imposition of silent periods, avoids or minimizes secretory fatigue at the terminals.

Mechanisms underlying facilitation and fatigue

The phenomena of facilitation and fatigue of the action potential–secretion coupling process are probably widespread and certainly occur, for instance at the neuromuscular junction where a number of explanations have been proposed (see Katz & Miledi, 1968; Brock & Cunnane, 1987; Nussinovitch & Rahamimoff, 1988). At the neurohypophyseal peptide secretory terminals the phenomena are probably multifacetted and interrelated and there is experimental evidence that: (1) the duration of action potentials arriving at the terminals is activity-dependent, (2) the conduction of action potentials into terminals is activity-dependent, (3) the entry of Ca^{2+} and the elevation of $[Ca^{2+}]_i$ are activity-dependent, and (4) the

extraterminal/extra-axonal extracellular K^+ concentration ($[K^+]_o$) is activity-dependent. This evidence will now be considered.

Action potential duration

The depolarizing phase of the action potential recorded at the soma of magnocellular neurones consists of both Na^+ and Ca^{2+} conductances (Mason & Leng, 1984; Bourque & Renaud, 1985*a*). The duration of the somatic action potential increases markedly as firing frequency increases up to about 20 Hz (Bourque & Renaud, 1985*b*).

In the terminal region, use of optical recording and potentiometric dyes has allowed demonstration of broadening of compound action potentials with increasing frequency of applied electrical impulses (Gainer, Wolfe, Obaid & Salzberg, 1986). Such spike broadening also occurs in invertebrate peptidergic terminals recorded electrophysiologically (Cooke, 1981). If action potentials are prolonged pharmacologically by blocking K^+ channels with, for instance, tetraethylammonium ions (TEA^+), release per pulse of OXT and AVP is greatly enhanced (Bondy *et al.* 1987; Hobbach, Hurth, Jost & Racke, 1988). This enhancement is inversely related to stimulus frequency suggesting that frequency facilitation and K^+ channel blockade share a common, saturable mechanism (Fig. 3; see Bondy *et al.* 1987). Facilitation may thus involve a frequency-dependent inactivation of K^+ channels and broadening of the depolarizing phase of each action potential. Intriguingly, K^+ channel blockade shifts the frequency-dependent secretion curve

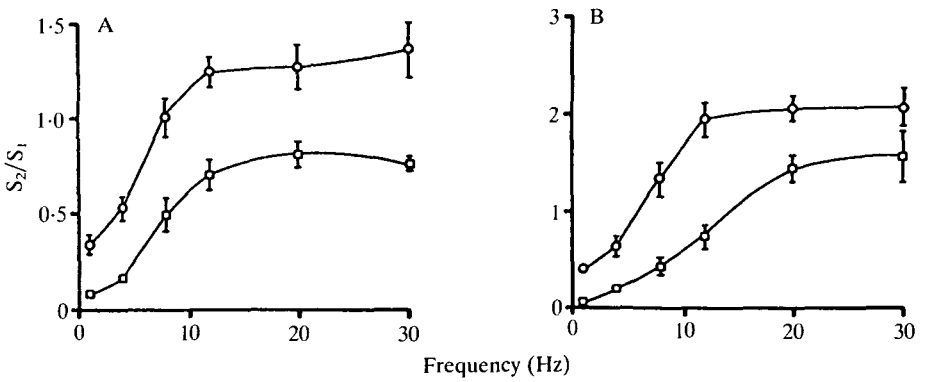

Fig. 3. Effects of pharmacological prolongation of action potentials with tetraethylammonium ions (TEA^+) on frequency-dependence of vasopressin (A) and oxytocin secretion (B). □, control; ○, TEA^+, 10 mmol l^{-1}. All stimuli consisted of a train of 600 pulses. Evoked release by the test stimulus (S_2) has been expressed as a ratio of that released by an initial standard stimulus delivered at 12 Hz (S_1). Note the different scales for A and B. Under control conditions the half-maximal release frequency for vasopressin is 6·9 Hz and for oxytocin is 12·3 Hz (compare with Fig. 1). In the presence of TEA^+, half-maximal release frequency is virtually unaltered for vasopressin (5·4 Hz) but is shifted significantly to the left for oxytocin (6·5 Hz). Values are means ± S.E., $N = 6$–12. Data drawn from Bondy, Gainer & Russell (1987).

to the left only in the case of OXT, suggesting differences in K^+ channel complement in the membranes of the two neurone types (Fig. 3). This difference itself may determine the differing frequency-dependencies of the release processes.

The converse explanation that fatigue in the release process with sustained stimulation is due to an *increase* in K^+ conductance and presumably a decreased action potential duration, has not yet been studied electrophysiologically. TEA^+, however, appears substantially to overcome fatigue of AVP release (Hobbach *et al.* 1988).

Action potential conduction

A further explanation of the mechanism of fatigue is the possibility that each action potential does not invade every secretory terminal membrane and that the probability of invasion occurring is dependent on the pattern of electrical activity. Evidence for failure of conduction with continuous stimulation of neurohypophyseal axons has been reported by measuring the amplitude of compound action potentials (Nordmann & Stuenkel, 1986). However, recordings of electrical activity of single terminals/axon branches has more recently demonstrated no significant failure of conduction at impulse frequencies of up to 20 Hz for 60 s (Dyball, Grossmann, Leng & Shibuki, 1988): conditions under which marked fatigue in release of AVP, in particular, will occur (Fig. 2). Furthermore, at the neuromuscular junction, intermittent release of transmitter is not due to intermittent conduction of the action potential into the nerve terminals (Brock & Cunnane, 1987). It is thus contentious whether conduction failure underlies fatigue of peptide release during sustained electrical activity.

Ca^{2+} entry and $[Ca^{2+}]_i$

If action potentials are indeed faithfully propagated into the terminal membranes then mechanisms underlying facilitation and fatigue of the release process may occur at this site. The experimental evidence here is limited. Measurement of uptake of $^{45}Ca^{2+}$ into the entire neurohypophysis during electrical stimulation has demonstrated both frequency-dependence and the importance of silent periods, i.e. avoidance of fatigue (Cazalis *et al.* 1985). This technique, however, does not discriminate between Ca^{2+} entry into axons, terminals or non-neuronal elements. More recent studies using suspensions of isolated neurohypophyseal terminals (neurosecretosomes) loaded with the Ca^{2+}-fluorescent indicator fura-2 have clearly shown that the rise in terminal $[Ca^{2+}]_i$, induced by electrical stimulation, is enhanced by a phasic pattern of stimulation (Fig. 4; Brethes, Dayanithi, Letellier & Nordmann, 1987). Data on the frequency-dependence of increases in $[Ca^{2+}]_i$ are as yet incomplete.

Both depolarization-evoked rises in $[Ca^{2+}]_i$ and secretion from neurosecretosomes appear to be entirely dependent on entry of extracellular Ca^{2+} through channels sensitive to a variety of well-characterized Ca^{2+} channel antagonists (Brethes *et al.* 1987; Cazalis *et al.* 1987*a*). Exocytosis is activated in permeabilized

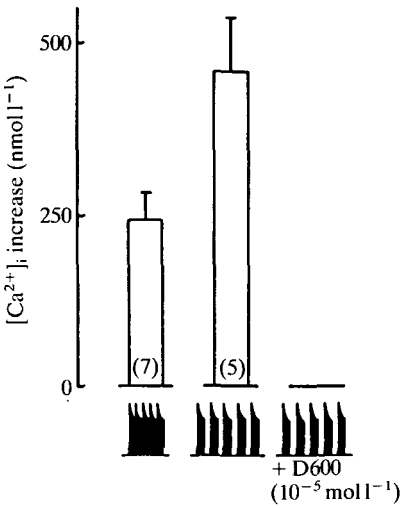

Fig. 4. Phasic pattern of electrical stimulation enhances evoked $[Ca^{2+}]_i$ increase in suspensions of isolated neurosecretory nerve terminals, neurosecretosomes. Neurosecretosomes, loaded with the Ca^{2+}-fluorescent indicator fura-2, were stimulated with five 25-s bursts of pulses similar to those generated by vasopressin neurones. Bursts consisted of an initial higher-frequency discharge (2 s at 25 Hz) followed by a plateau at lower frequency (see text). Imposition of silent periods of 180 s between bursts resulted in much greater increases in $[Ca^{2+}]_i$ than if bursts were delivered without silent periods. Evoked rises in $[Ca^{2+}]_i$ were prevented by the Ca^{2+} channel antagonist, D600. Values are means ± S.E., *N* values are given in parentheses. Data are taken from Brethes, Dayanithi, Letellier & Nordmann (1987).

neurosecretosomes by 10^{-6} mol l^{-1} Ca^{2+} (Cazalis *et al.* 1987*b*). Using patch-clamp recording, channels conducting Ca^{2+} have recently been described in neurosecretosomes (Lemos & Nordmann, 1986) and putative neurohypophyseal terminals (Mason & Dyball, 1986). In the latter situation some evidence was obtained that channel openings were more frequent following trains of stimuli, perhaps reflecting some facilitatory mechanism.

Extracellular $[K^+]_o$

A further set of experimental findings suggests that activity-related changes in $[K^+]_o$ exert an important influence over action potential–secretion coupling. Using K^+-sensitive microelectrodes, application of electrical impulses to axons in the neural stalk has been shown to lead to increases in $[K^+]_o$ in the neurohypophysis up to a concentration of about 15 mmol l^{-1}. These increases are dependent upon both duration and frequency of stimulation (Fig. 5A; Leng & Shibuki, 1987). The increase in $[K^+]_o$ during electrical stimulation is thought to derive from outward movement of K^+ from axons during the repolarizing phase of each action potential. If the neurohypophysis is bathed in media containing 15 mmol l^{-1} K^+, electrically induced release of peptides per pulse at relatively low frequency (6 Hz) is greatly facilitated (Fig. 5B; Leng, Shibuki & Way, 1988). At $[K^+]_o$ above

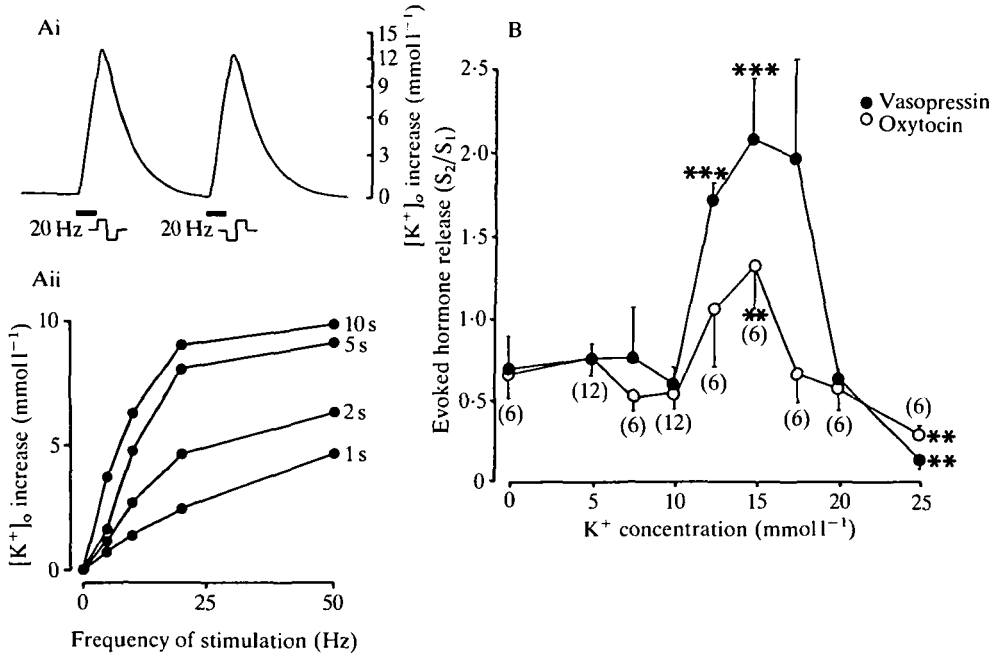

Fig. 5. (A) Electrical stimulation of neurohypophyseal axons increases $[K^+]_o$ recorded with K^+-sensitive electrodes in the neurohypophysis. (i) Stimulation at 20 Hz for 5 s (bars) increases $[K^+]_o$ up to a maximum of 12–15 mmol l^{-1}; (ii) maximal $[K^+]_o$ obtained at different durations and frequencies of stimulation. Drawn from Leng & Shibuki (1987). (B) Effects of varying medium $[K^+]$ on peptide release from the neurohypophysis in response to electrical stimulation for 5 min at 6 Hz. Secretion of both peptides was enhanced when medium $[K^+]$ was increased to 15 mmol l^{-1} but was inhibited above 20 mmol l^{-1}. ** $P < 0.01$, *** $P < 0.001$ *vs* release evoked in 5 mmol l^{-1} K^+-containing media. Values are means ± S.E., *N* values are given in parentheses. Reproduced from Leng, Shibuki & Way (1988).

20 mmol l^{-1} an inhibitory influence on secretion is seen which may be due to inability of axonal membranes to propagate the action potential. Indeed, stimulus pulses applied at 30 Hz, which itself evokes a large rise in $[K^+]_o$, become less efficient in releasing peptide when medium $[K^+]$ is raised by only 5–10 mmol l^{-1} (Leng *et al.* 1988). Thus, activity-related changes in $[K^+]_o$ may be involved in both facilitatory and fatigue mechanisms of action potential–secretion coupling. The exact relationship of changes in $[K^+]_o$ with the measured changes in action potential duration and propagation are not yet clear (see above).

Taken together, the experimental evidence provides an as yet incomplete picture but indicates that facilitation and fatigue in peptide release are likely to involve mechanisms operating both along the axons prior to terminal depolarization and at the level of the terminals themselves. This conclusion would not seem surprising if one takes the view that the terminals are not highly specialized sit but merely extensions of the axonal membrane, possessing similar properties.

Receptor-mediated modulation

Receptors mediating influences upon the presynaptic element of the mammalian synapse have been known for a number of years (see Starke, 1981). Secretory terminals represent the analogous site for endocrine neurones. Among a number of neuroactive substances shown to modify secretion of OXT and AVP at the level of the neurohypophysis (Leng & Bicknell, 1987), only the actions of opioids and noradrenaline have been characterized. The mechanisms by which these substances modulate the secretory process appear to differ, opioids acting directly on the terminals and noradrenaline acting indirectly, possibly *via* glial cells. These mechanisms will be described and their physiological functions considered.

Direct modulation by opioid receptors

Uncoupling of the electrical message generated at the cell bodies from release of secretory product by an inhibitory effect of opioids in the neurohypophysis was first suggested to occur during reflex release of OXT at milk ejection (Clarke, Wood, Merrick & Lincoln, 1979). This inhibitory action was confirmed *in vitro*, shown to be mediated by opioid peptides co-produced in the magnocellular neurones and to be relatively selective for OXT (see Bicknell, 1985; Bondy *et al.* 1988). When the actions of opioids are prevented by opioid receptor antagonists or antibodies to opioid peptides, the amounts of OXT released from the isolated neurohypophysis per applied stimulus pulse are approximately doubled (Bicknell & Leng, 1982; Maysinger *et al.* 1984). Only opioids active at the κ-opioid receptor subtype are effective and this is the predominant type of opioid receptor found in the neurohypophysis (Herkenham, Rice, Jacobson & Rothman, 1986; Zhao, Chapman & Bicknell, 1988*a*).

Opioid peptides derived from pro-dynorphin appear to be selective endogenous ligands for κ-receptors. Since dynorphin peptides in the neurohypophysis are produced exclusively in the AVP neurones, it is speculated that opioid modulation of OXT terminals occurs by 'cross-inhibition' from dynorphins secreted by neighbouring AVP neurones (Summy-Long *et al.* 1984), although some difficulties remain with this hypothesis (Zhao, Chapman, Brown & Bicknell, 1988*c*).

κ-Receptors are present on neurosecretosomes and act directly at this site to inhibit secretion in response to depolarization, in this instance induced by elevated-K^+ medium (Fig. 6; Zhao *et al.* 1988*b*). In other neurones κ-receptors at the cell soma inhibit the plasma membrane Ca^{2+} conductance (North, 1986). Such a mechanism, operating at the terminal, might thus be expected to reduce Ca^{2+} entry and the rise in $[Ca^{2+}]_i$ in response to invasion by the depolarizing potential. Opioid receptor antagonists are reported to increase $^{45}Ca^{2+}$ incorporation into the intact neurohypophysis but a more direct demonstration of κ-receptor effects on $[Ca^{2+}]_i$ has not yet been obtained (Nordmann, Dayanithi & Cazalis, 1985).

Previous experiments defining facilitation and fatigue of the release process have been performed under conditions where selective suppression of OXT

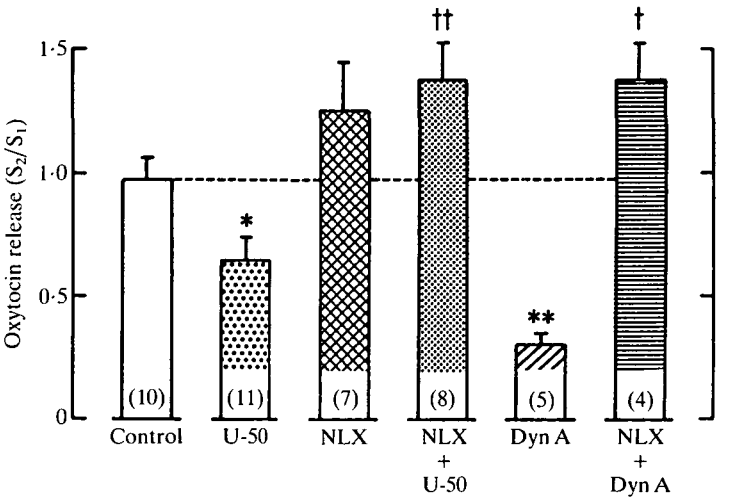

Fig. 6. Activation of κ-opioid receptors on oxytocin (OXT) nerve terminals inhibits depolarization-evoked release of peptide. Neurosecretosomes retained on filters in a perfusion system received two periods of depolarization with elevated K^+-containing media (5 min, 60 mmol l^{-1} K^+). Opioid compounds were included during the second period of depolarization. Release of OXT evoked by the second stimulation (S_2) was expressed as a ratio of that evoked by the initial stimulation in the absence of test compounds (S_1). Inclusion of the κ-receptor agonists U-50,488H (10^{-6} mol l^{-1}) and dynorphin A,1-13 (Dyn A, 10^{-6} mol l^{-1}) strongly inhibited OXT release. Inhibitions were prevented by the competitive opioid receptor antagonist naloxone (NLX, 10^{-5} mol l^{-1}). * $P<0{\cdot}05$, ** $P<0{\cdot}01$ *vs* control S_2/S_1, † $P<0{\cdot}05$, †† $P<0{\cdot}01$ *vs* agonist without NLX. Values are means ± S.E., *N* values are given in parentheses. Data from Zhao, Chapman & Bicknell (1988*b*).

secretion by intrinsic opioids is taking place. Is it possible that selective opioid modulation underlies the different frequency-facilitation and fatigue characteristics of OXT and AVP terminals? When opioid actions are prevented, the frequency–response curve for OXT release is indeed shifted to a lower range (Bondy *et al.* 1988). This effect is thus similar to that seen upon blocking K^+ channels with TEA^+ (Fig. 3). TEA^+ will also prevent opioids from inhibiting OXT secretion in the neurohypophysis (Racke *et al.* 1987). These findings suggest that opioids may act selectively on OXT terminals to increase K^+ conductance and perhaps shorten the duration of the action potential to reduce its efficacy in eliciting release at the terminals. However, this suggestion does not concur with a direct action of κ-opioids on Ca^{2+} conductance. The differential time course in fatigue of the release process between OXT and AVP terminals is not eliminated when opioid actions are prevented (Bicknell, 1988).

Indirect modulation via *receptors on glia*

The specialized astroglia of the neurohypophysis, the pituicytes, send processes around the neurosecretory axons and along the capillary basal lamina between

putative secretory terminals and the perivascular space. The intimate anatomical relationship between the pituicytes and neurosecretory elements has long suggested an involvement of these cells in the neurosecretory process. This idea receives support from quantitative ultrastructural studies in which a remarkable morphological plasticity of the pituicytes has been described in association with conditions of elevated secretion of neurohypophyseal hormones. Thus, during progressive dehydration and at parturition and throughout lactation, pituicyte processes withdraw from around neurosecretory elements and from the capillary basal lamina (Tweedle & Hatton, 1980, 1982, 1987). This reorganization results in a greater number of secretory terminals aligned along the capillary and raises the prospect that these are adaptive changes to allow greater release of hormones into the circulation. The signal(s) for morphological alterations in pituicytes is not known. Astrocytes elsewhere in the brain possess a wide range of receptors for neuroactive substances (Murphy & Pearce, 1987) which may potentially exert effects on cell morphology. Of particular interest are β-adrenergic receptors.

Cultured neonatal astrocytes from a number of brain regions possess β-adrenergic receptors which, upon activation with noradrenaline, stimulate rapid transformation from a flattened, irregular morphology to a stellate, process-bearing morphology by cytoplasmic withdrawal to the perinuclear area (Narumi, Kimelberg & Bourke, 1978). The neurohypophysis receives a noradrenergic innervation and contains β-adrenergic receptors which appear not to be present on neural elements (Bunn, Hanley & Wilkin, 1986). Our own studies indicate that pituicytes in culture are also stimulated to adopt a process-bearing morphology by noradrenaline acting on β-receptors (Bicknell, Hatton & Mason, 1986). Current studies are aimed at determining whether noradrenaline regulates pituicyte morphology *in situ*.

When β-adrenergic receptors are acutely activated in the intact neurohypophysis, electrically evoked secretion of OXT and AVP is enhanced (Zhao *et al.* 1988*c*). This enhancement does not occur when neurosecretosomes are treated with β-receptor agonists (B.-G. Zhao & R. J. Bicknell, unpublished data). Thus, acute β-receptor modulation of action potential–secretion coupling is probably occurring *via* the pituicytes. Although significant morphological changes in these cells may perhaps take place over the time course examined (15 min) the acute effects may be mediated in other ways. One possibility is that the pituicytes, like glia elsewhere in the brain, act to buffer rises in extracellular K^+ concentration occurring during neuronal activity. Modification by β-receptors of pituicyte membrane K^+ permeability or K^+ pumping could thus result in altered efficiency in the coupling of action potentials to secretion at the terminals, as described above. This indirect receptor-mediated mechanism will require further experimental testing.

Physiological significance of receptor-mediated modulation

At first sight it seems wasteful that the exquisite neuronal organizations which regulate the electrical activity of neurosecretory neurones should be overriden

downstream by inputs to the terminal region. Presumably this site represents a further level of control. This certainly appears to be the case for opioid modulation of OXT secretion. In 'normal' animals and in man, the opioid mechanism does not appear active, but can be switched on in a number of physiological and experimental situations. Thus, in female rats a progressive increase in opioid input to OXT neurones is seen during pregnancy and is greatest during parturition (Bicknell *et al.* 1988; Hartman, Rosella-Dampman, Emmert & Summy-Long, 1986). The purpose of this input is not fully understood but it appears to be involved in the spacing of deliveries (Leng *et al.* 1985) and perhaps in allowing the suspension of parturition upon environmental disturbance (Leng *et al.* 1987). It should be pointed out that sites of opioid action other than at the secretory terminals may be operating under these circumstances. Opioid input to OXT neurones is also activated by osmotic stimuli when secretion of AVP and co-existing dynorphins is enhanced (Summy-Long *et al.* 1984). Since electrical activity of the OXT neurones is not affected this mechanism is clearly operating at the nerve terminals, perhaps to conserve stores of OXT preferentially (Shibuki, Leng & Way, 1988).

Conclusions

Optimal secretion per action potential, of peptide products from the terminals of endocrine neurones, appears to be achieved by generation of particular patterns of electrical activity. These patterns have profound consequences for a number of ionic mechanisms at the distal axons and terminals which affect shape and propagation of the action potential and the size of the inward Ca^{2+} flux signal for exocytosis. Patterns of electrical activity are characteristic for OXT and AVP neurones and are matched to the differing modes of secretion of the hormones required for their different biological roles. Further modulation of action potential–secretion coupling can be mediated by receptors for other neuroactive substances which represent a further level of homeostatic control. Receptor-mediated influences may utilize similar ionic mechanisms in neuronal membranes or perhaps in surrounding glial cells.

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