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DORSOMEDIAL MEDULLA STIMULATION ACTIVATES RAT SUPRAOPTIC OXYTOCIN AND VASOPRESSIN NEURONES THROUGH DIFFERENT PATHWAYS

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SUMMARY

1. This study utilized retrograde anatomical tracer techniques and *in vivo* extracellular electrophysiological studies to examine caudal ventrolateral and dorsomedial medulla afferents to supraoptic nucleus neurosecretory neurones in male Long–Evans rats.

2. In one series of experiments, pentobarbitone-anaesthetized animals were subjected to ventral exposure of the hypothalamus and rhodamine-tagged latex microspheres (0·05–0·2 μl) were injected into one supraoptic nucleus. Following perfusion with paraformaldehyde–glutaraldehyde 18–24 h later, cell counts were obtained of rhodamine- and/or catecholamine-labelled neurones in the caudal ventrolateral and dorsomedial medulla both ipsi- and contralateral to the injection site.

3. In the caudal ventrolateral medulla, each injection labelled fewer than 15% of the catecholaminergic neurones; with small injections, most (68–100%) of the rhodamine-labelled neurones also displayed catecholamine histofluorescence. In the caudal nucleus tractus solitarii, one-half to one-third as many rhodamine-labelled cells were observed, but a higher percentage (13–100%) of these were non-catecholaminergic.

4. Extracellular recordings were obtained from antidromically identified supraoptic neurones classified as vasopressin ($n = 106$) or oxytocin ($n = 26$) secreting. Single cathodal pulses (0·2 ms duration, 0·02–0·08 mA) applied in the caudal half of the ipsilateral nucleus tractus solitarii evoked a transient (30–50 ms) activation of 63% of both vasopressin- and oxytocin-secreting neurones. Mean latencies ($\pm$S.E.M.) for vasopressin and oxytocin cells were 49·8 $\pm$ 1·0 and 46·5 $\pm$ 2·4 ms respectively; these were not significantly different. Similar responses were noted to contralateral stimuli applied to four vasopressin and two oxytocin cells.

5. Vasopressin neurones activated by caudal nucleus tractus solitarii stimulation displayed similar patterns of response to stimulation in the caudal ventrolateral medulla. However, latencies from the nucleus solitarius (mean 47·6 $\pm$ 1·4 ms; $n = 59$) were significantly longer ($P < 0·05$) than from the ventrolateral medulla (41·5 $\pm$ 2·0 ms; $n = 17$). In eight out of eleven vasopressin neurones tested, interruption of synaptic transmission through the ventrolateral medulla reduced or abolished the

caudal nucleus tractus solitarii-evoked excitation but had no effect on their response to baroreceptor activation. This manoeuvre affected zero out of five oxytocin cells similarly excited by nucleus solitarius stimulation.

6. These observations indicate that visceral input mediated through the nucleus tractus solitarii is transmitted differentially to supraoptic vasopressin- and oxytocin-secreting neurones.

INTRODUCTION

The hypothalamic supraoptic nucleus (SON) contains magnocellular neurosecretory cells that synthesize either vasopressin or oxytocin for release from their axon terminals in the neurohypophysis. In the rat, the anatomical organization and transmitter content of afferents to SON neurones has been the subject of several investigations (Zaborszky, Leranth, Makara & Palkovits, 1975; McNeil & Sladek, 1980; Sawchenko & Swanson, 1981, 1982, 1983; Swanson & Sawchenko, 1983; Tribollet, Armstrong, Dubois-Dauphin & Dreifuss, 1985; see Renaud, 1988, for review). Of particular interest is an intense catecholamine terminal innervation (Carlsson, Falck & Hillarp, 1962; Fuxe, 1965) that is preferentially concentrated around the vasopressin neurones (McNeil & Sladek, 1980; Swanson, Berod, Hartman, Helle & Van Orden, 1981; Cunningham & Sawchenko, 1988), and arises primarily from the A_1 noradrenergic neurones in the caudal ventrolateral medulla (CVLM) (Sawchenko & Swanson, 1981, 1982, 1983; McKellar & Loewy, 1982). An additional, albeit less dense, catecholamine input to SON arises from the A_2 neurones in the caudal nucleus tractus solitarii (Tribollet *et al.* 1985; Cunningham & Sawchenko, 1988). Consistent with an important facilitatory role for these ascending catecholaminergic projections on vasopressin neurosecretory neurones are measurements of plasma vasopressin levels consequent to brain stem lesions or stimulation (e.g. Lightman, Todd & Everitt, 1984; Yamane, Nakai, Yamamoto, Nemeda & Ogino, 1984; Blessing & Willoughby, 1985*a*, *b*; Head, Quail & Woods, 1987) and the responsiveness of SON neurones to ventrolateral medulla stimulation (Day & Renaud, 1984). However, our initial electrophysiological observations in the rat SON (Raby & Renaud, 1987) indicated that caudal nucleus tractus solitarii (CNTS) stimulation consistently excited a majority of *both* vasopressin and oxytocin neurones, contrasting sharply with previous data indicating a selective excitation of vasopressin cells following CVLM stimulation (Day & Renaud, 1984). These differences prompted us to evaluate both the anatomy and electrophysiology of the CNTS linkage to SON more closely and to compare this input with the CVLM innervation. Anatomical studies utilized retrograde transport of rhodamine microspheres from the SON to determine the relative ipsi- and contralateral input to SON from CNTS and CVLM and histofluorescence to distinguish between catecholamine and non-catecholaminergic retrogradely labelled neurones. Our electrophysiological studies confirmed the excitatory influence of CNTS stimulation on the excitability of 63% of both vasopressin and oxytocin neurones, and determined that the CNTS-evoked excitation of vasopressin, but not oxytocin, neurones is mediated through the ventrolateral medulla A_1 cell group.

METHODS

Retrograde tracer and histofluorescence studies

Male Long–Evans rats were initially anaesthetized with intraperitoneal sodium pentobarbitone (50 mg/kg) and maintained on intravenous supplements of 2–4 mg as required. Heart rate was monitored continuously and body temperature was maintained at 37 °C. After tracheal intubation, a transpharyngeal approach that included removal of a portion of the trigeminal nerve was utilized to expose the ventral surface of the hypothalamus and area of the SON. In each of six animals, 0·05–0·2 μl of a suspension of rhodamine-conjugated latex microspheres (0·2–0·5 μm; Lumaflor, New City, NY, USA) was delivered directly into one nucleus by pressure ejection through glass micropipettes with a tip diameter of 25–50 μm. Animals were maintained under pentobarbitone anaesthesia for a further 20–28 h and perfused transcardially with a mixture of 4% formaldehyde–0·5% glutaraldehyde so as to permit subsequent visualization of catecholamine histofluorescence (Furness, Heath & Costa, 1978). The brains were sectioned at 40 mm on a vibrotome, desiccated, cover-slipped in paraffin oil and examined in a Nikon fluoresence microscope using a V-1A filter for catecholamines and a G-1B filter for rhodamine visualization. In sections caudal to the obex, singly and doubly labelled neurones in the CVLM and CNTS were counted and tabulated (Table 1).

Electrophysiological investigations

For electrophysiological experiments, the transpharyngeal dissection was extended to expose the pituitary and the ventral surface of the pons and the medulla. A bipolar nichrome electrode (tip separation, 1·0 mm; exposure 0·7 mm) connected to an isolated stimulator (pulse duration, 200 μs, intensities of 200–800 μA) was positioned in the neural lobe in order to antidromically activate SON neurosecretory neurones (see below). A second glass-insulated tungsten stimulating electrode (tip diameter, 25 μm; exposed length, 50 μm) was directed through the medulla for activation of the CNTS (cathodal pulses, 0·2 ms duration; intensities of 0·02–0·08 mA). At the highest intensities of CNTS stimulation utilized, maximum current spread under 400 μm would be anticipated (Bagshaw & Evans, 1976). A cannula placed in the femoral artery monitored mean arterial blood pressure. The femoral vein was cannulated for brief infusions of an α-agonist (metaraminol, 2–10 μg) so as to transiently elevate arterial pressure by 70–100 mmHg, sufficient to activate peripheral baroreceptors (see below).

Extracellular recordings from SON neurones were obtained with glass micropipettes (15–30 MΩ) filled with 3·0 M-sodium acetate, amplified, band-pass filtered, displayed on an oscilloscope and fed through a window discriminator to a PDP 11/23 computer programmed to acquire spike train data with a resolution of 1 ms. Neurosecretory cells were first localized by antidromic activation; criteria included constant latency, all-or-none responses at threshold, ability to follow two stimuli presented at 5 ms intervals and evidence of collision-cancellation of antidromic responses with spontaneous spikes occurring within one antidromic latency of the stimulus. Earlier studies (Harris, 1979; Day, Ferguson & Renaud, 1984; Day & Renaud, 1984) have suggested that SON neurones may be subdivided according to whether they fire continuously or in phasic bursts, and whether their on-going activity is transiently suppressed during activation of peripheral baroreceptors. In our experience (Day *et al.* 1984), most phasic SON neurones as well as a population of continuously firing cells are sensitive to baroreceptor activation. In view of the strong correlation between phasic firing and vasopressin immunoreactivity noted in *in vitro* studies (Yamashita, Inenaga, Kawata & Sano, 1983; Cobbett, Smithson & Hatton, 1986), we have tentatively classified those cells that are baroresponsive and/or display firing as vasopressin neurones. The SON of male rats also contains a population of continuously firing neurosecretory neurones that are non-baroresponsive; since earlier studies (Renaud, Tang, McCann, Stricker & Verbalis, 1987) have shown that such cells show a marked increase in activity coincident with a selective rise in plasma oxytocin, we have classified these cells as oxytocin neurones. In many rats, the coincidental anatomical disposition of the middle and anterior cerebral arteries prevented access to the rostral half of the SON, which contains a larger number of oxytocin-immunoreactive neurones. The bias for vasopressin-immunoreactive neurones in the caudal and ventral SON (Swanson & Sawchenko, 1983) is reflected in the smaller sample of putative oxytocin neurones detected in this study (see Results).

Responses to CNTS stimulation were assessed according to patterns detected in peristimulus histograms: a 30% stimulus-induced increase or decrease in excitability was required to classify a response as excitation or inhibition respectively. Latencies (resolution, 1 ms) were estimated by first determining the average spike number per bin for a duration of 100 ms preceding the CNTS pulse. The onset of a sustained response was set at the first bin following the pulse that contained a number of spikes 30% greater than baseline. The end of the response, estimated in a similar way, was set at the first bin following the onset of the response with a number of spikes less than 30% over baseline. The duration of the response was calculated as the difference between the two points.

In five experiments the contribution of the CVLM to the CNTS-evoked response was assessed as follows. Prior to electrophysiological recordings in SON, the contralateral CVLM was injected with kainic acid (50 μM, 0·2–0·4 μl) to destroy the A_1 cell group. After identification of the nucleus tractus solitarii (NTS)-evoked response of a particular SON cell, either 20 μM-muscimol ($n = 2$) or 10 mM-γ-aminobutyrate (GABA; $n = 3$) were infused by pressure ejection in the ipsilateral A_1 region to transiently alter synaptic transmission, and attenuation of the CNTS-evoked response, if any, was monitored. The addition of Eosin Y to the infused solutions facilitated determinations of the extent of the kainic acid lesion and muscimol or GABA infusions in histological section.

At the termination of each electrophysiological experiment, a brief 10 μA current was passed through the tungsten stimulating electrode to produce a small lesion in the dorsomedial medulla. Following fixation and tissue sectioning, the location of this lesion was subsequently verified with respect to the position of the A_2 catecholaminergic neurones, as revealed by the Faglu histofluorescence method (Furness *et al.* 1978).

RESULTS

Retrograde tracer studies

All six rhodamine-conjugated microsphere injections were centred on the SON. Two experiments where injections were strictly confined to the cytoarchitectural boundaries of the nucleus (Plate 1*A* and Fig. 1) and a third experiment where the injection extended marginally beyond the nucleus (intermediate injection size in Table 1) served to identify sites projecting only to the SON. These injections retrogradely labelled cells in several forebrain regions including the immediate perinuclear zone (Fig. 1*A*), median preoptic nucleus, organum vasculosum of the lamina terminalis and subfornical organ (not illustrated). In the hindbrain, retrogradely labelled cells were observed both ipsilateral and contralateral to the site of injection within two catecholamine histofluorescent sites in the caudal medulla: the ventrolateral region containing the A_1 neurones, and the A_2 region of the nucleus tractus solitarii (Table 1; also Plate 1*C* and *D*). In three other experiments, where injections clearly extended beyond the boundaries of the SON (e.g. Plate 1*B*; Fig. 1), retrogradely labelled neurones were observed in additional forebrain and hindbrain structures including the nucleus of the diagonal band of Broca, the ventral subiculum and dorsal parabrachial nucleus. Since the dendrites of SON neurones remain essentially confined to the anatomical boundaries of the nucleus (Randle, Bourque & Renaud, 1986*b*), these latter observations infer that these structures preferentially innervate the SON perinuclear region rather than the nucleus *per se*. This project was focused on the retrogradely labelled neurones in the caudal medulla.

All six experiments resulted in rhodamine-labelled neurones in the CVLM and CNTS. Labelled neurones (Fig. 2; also Plate 1*C* and *D*) were observed both ipsilateral and contralateral to the site of injection in an approximate ratio of 3:1, and included both catecholamine (double-labelled) and non-catecholamine (rhodamine-labelled only) cells (Table 1). In any individual experiment only a fraction (i.e. less than 15%)

of the catecholamine neurones was labelled with rhodamine. Cell counts from small and intermediate injections, which reflect most accurately inputs to the nucleus itself rather than the perinuclear zone, suggest that the input to SON from ipsilateral and contralateral CVLM is almost exclusively (> 90%) catecholaminergic (i.e. from the

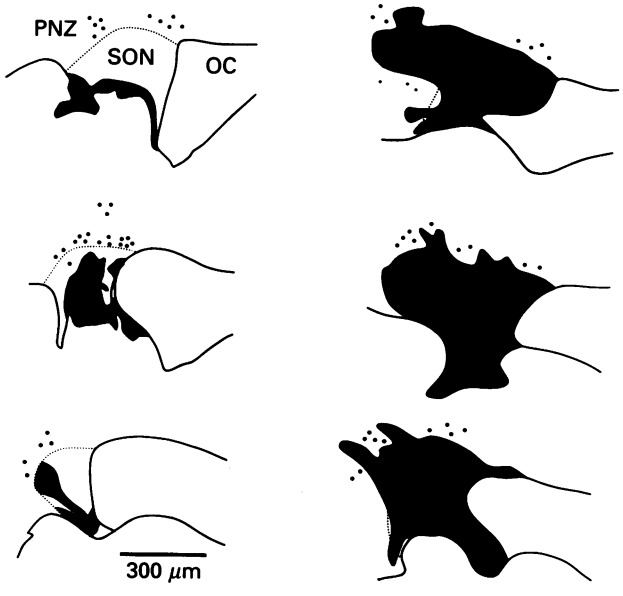

Fig. 1. Schematic coronal sections (at three intervals of 150 μm each) illustrate the disposition of rhodamine latex-bead injections (dark areas) within the supraoptic nucleus (SON). On the left, the bead distribution in the smallest injection of the six experiments; on the right, bead distribution in the largest injection (see Table 1). Fine dots represent the location of retrogradely labelled cells in the perinuclear zone (PNZ). OC, optic chiasm.

A_1 cell group). Although even fewer cells in CNTS are labelled, the CNTS input to SON contains relatively more neurones in the non-catecholaminergic category. Within the CNTS, most retrogradely labelled neurones, whether doubly fluorescent (for rhodamine and catecholamines) or not, were confined to the medial part of the NTS and the nucleus commissuralis (Fig. 2). No retrogradely labelled cells were detected in the dorsal motor nucleus of the vagus.

Electrophysiology

Extracellular data were obtained from a total of 106 vasopressin and 26 oxytocin SON neurones. Latencies for antidromic activation from the neural lobe stimulus ranged between 8 and 28 ms. Another twenty-four neurosecretory neurones could not be readily classified into either group owing to inconsistent responses to blood pressure manipulation. The data in Table 2, based on peristimulus time histograms (Figs 4–6) indicate that a majority of both vasopressin and oxytocin neurones displayed an increase in excitability to ipsilateral CNTS stimulation. The most effective sites for eliciting a response at the lowest current intensities (i.e. < 10 μA) coincided with stimulation positions located among catecholamine neurones in the caudal portion of the NTS (Fig. 3). Latencies for all responses varied between 40 and

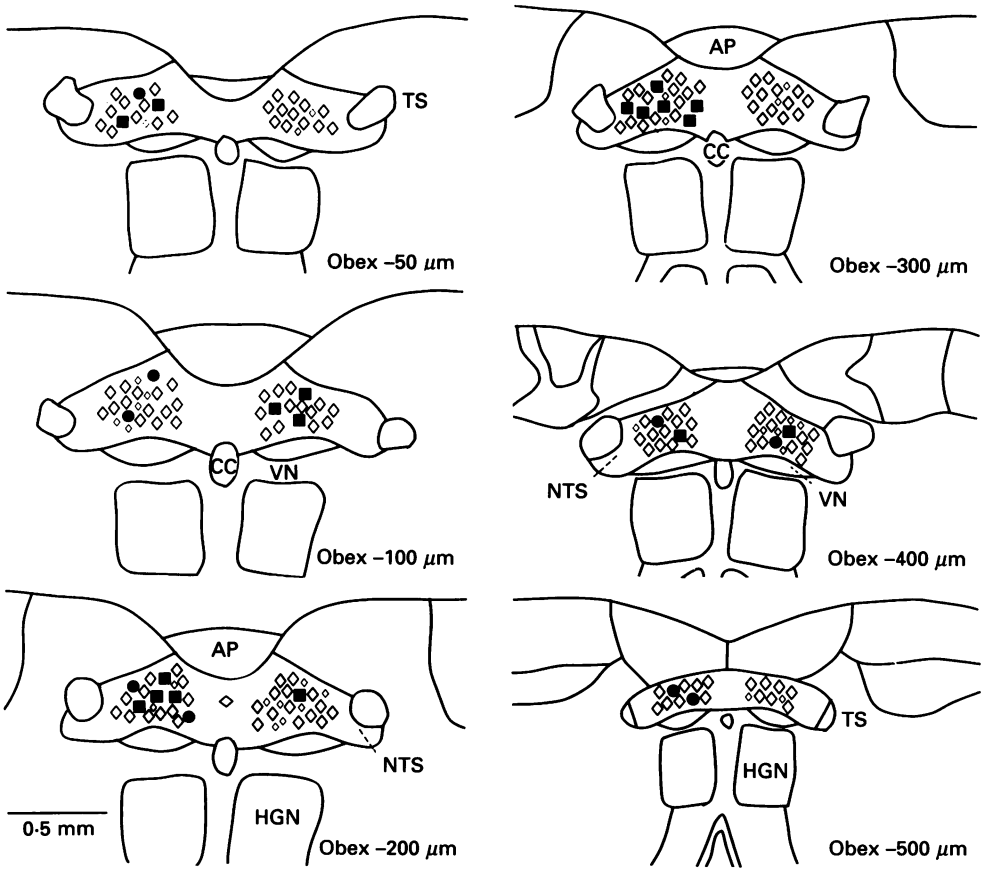

Fig. 2. Schematic coronal sections of the dorsomedial medulla at several levels caudal to the obex illustrate the disposition (but not the magnitude) of retrogradely labelled neurones. The left is ipsilateral to the large injection of rhodamine microspheres into the SON referred to in Table 1. ◇, catecholamine fluorescent cells; ●, rhodamine fluorescent cells; ■, neurones demonstrating both markers. Abbreviations: AP, area postrema; CC, central canal; NTS, nucelus tractus solitarii; HGN, hypoglossal nucleus; VN, vagal nucleus; TS, tractus solitarius.

TABLE 1. Numbers of caudal nucleus tractus solitarii (CNTS) and caudal ventrolateral medulla (CVLM) neurones that fluoresce only for catecholamines (C), only for rhodamine (R) or for both (C and R)

		Ipsilateral			Contralateral		
Site	Injection site	C	R	C and R	C	R	C and R
CNTS	Large	381	23	32	322	6	10
	Intermediate	270	2	13	305	6	0
	Small	310	4	10	300	2	2
CVLM	Large	455	45	69	424	15	25
	Intermediate	417	1	27	382	0	6
	Small	415	0	28	407	8	17

Listed are data both ipsilateral and contralateral to the site of large, intermediate and small latex-bead injections into one supraoptic nucleus; the small injection remained strictly within the anatomical boundaries of the nucleus.

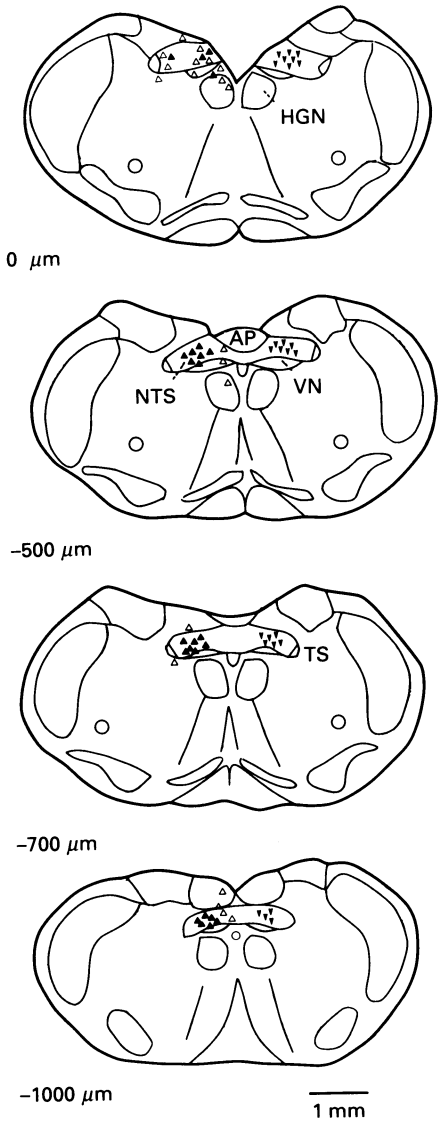

Fig. 3. Schematic series of coronal sections through the caudal medulla. Symbols (inverted triangles) on the right depict the location of catecholamine fluorescent neurones in the nucleus tractus solitarii (NTS). Filled symbols on the left designate the sites of electrical stimulation that elicited excitatory responses from SON neurones; open symbols represent ineffective sites. Abbreviations: AP, area postrema; VN, vagal nucleus; HGN, hypoglossal nucleus; TS, tractus solitarius. Values to the left indicate distance from obex.

60 ms. The mean latency for each cell type was similar: $49{\cdot}8 \pm 1{\cdot}0$ for vasopressin cells and $46{\cdot}5 \pm 2{\cdot}6$ ms for oxytocin cells. Effects lasted 30–50 ms (mean, $38 \pm 1{\cdot}2$ ms) except in eight of fifty-six vasopressin and one of fourteen oxytocin cells where the response was prolonged (mean, 147 ± 8 ms). Electrical stimulation of the contra-

lateral CNTS also produced excitatory responses of similar latencies and durations in two of four vasopressin and one of two oxytocin neurones thus corroborating the anatomical evidence for a contralateral projection from the CNTS to the SON.

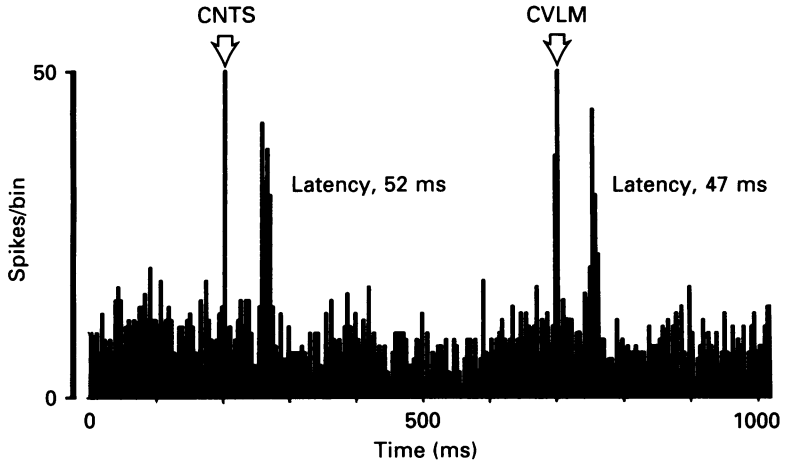

Fig. 4. Peristimulus histogram from one supraoptic neurone reflects the increase in excitability recorded from all three vasopressin cells following stimulation (arrow) in the caudal nucleus tractus solitarii (CNTS) *and* caudal ventrolateral medulla (CVLM). Latencies as determined with a 1 ms resolution were consistently shorter for the CVLM-evoked response.

TABLE 2. Data from electrophysiological experiments on supraoptic nucleus neurones

		Response to CNTS stimulation (%)		
Cell type	No. of cells	Excitation	Inhibition	No effect
Vasopressin	106	63	16	21
Oxytocin	26	63	6	31
Unclassifiable	24	67	25	8

Neurones are classified where possible as vasopressin- or oxytocin-secreting. Responses to ipsilateral CNTS stimulation based on peristimulus histogram patterns are excitatory, inhibitory or 'no effect'.

The results in Table 2 reveal no preferential influence of the CNTS stimulus on vasopressin *versus* oxytocin neurones, in sharp contrast to the selective excitation of vasopressin neurones which follow CVLM stimulation (Day *et al.* 1984; Day & Renaud, 1984). However, the latencies of response for vasopressin cells were different, and gave a mean of $47{\cdot}6 \pm 1{\cdot}4$ for fifty-nine cells tested with CNTS stimulation compared with $41{\cdot}5 \pm 2{\cdot}0$ ms for seventeen cells tested with CVLM stimulation. When the same vasopressin neurones ($n = 3$) were tested with a sequential stimulus delivered in CNTS and CVLM, the latency of the CVLM-evoked response was noted to be 5–7 ms shorter than the CNTS-evoked excitatory response (Fig. 4) thereby raising the possibility that an anatomical projection from NTS to the ventrolateral medulla (VLM) (see Sawchenko, Cunningham & Levin, 1987) might

relay indirectly the CNTS-evoked response. Therefore, in five experiments, the contralateral VLM was initially lesioned with an injection of kainic acid to obliterate contralateral input to SON. Caudal nucleus tractus solitarii-evoked responses of individual neurones were then examined for any influence of transient synaptic

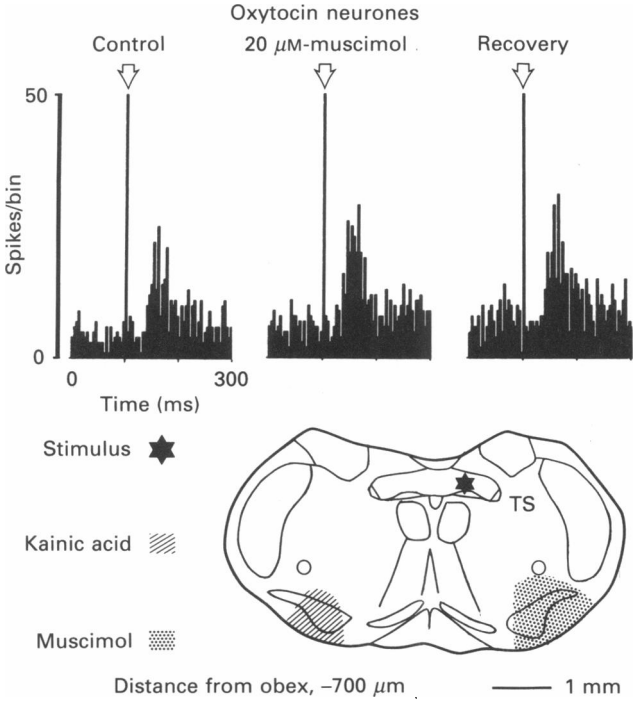

Fig. 5. Peristimulus histograms representative of data from all five oxytocin-secreting supraoptic neurones reveal excitations to CNTS stimulations when tested under control conditions (kainic acid lesion in the contralateral ventrolateral medulla). In this particular experiment, muscimol was injected into the ipsilateral ventrolateral medulla. Note that this manoeuvre did not affect the evoked response. TS, tractus solitarius.

interruption in the ipsilateral VLM by injections of GABA or a $GABA_A$ agonist muscimol (see Methods). While this procedure did not alter the CNTS-evoked response of any of five oxytocin neurones tested (Fig. 5), there was a dramatic but reversible reduction in the CNTS-evoked response from eight of eleven vasopressin neurones tested (Fig. 6). However, it is noteworthy that these manoeuvres did not alter their depressant response to activation of peripheral baroreceptors during a brief metaraminol-induced rise in arterial pressure. These observations support the proposal that the CNTS-evoked excitation of vasopressin neurones is mediated through the VLM whereas the influence of CNTS on oxytocin cell excitability reflects activation of a direct input to SON.

DISCUSSION

The neurones forming the various subnuclei of the CNTS collectively serve as a primary central integration centre for visceral information of cardiovascular,

respiratory and gastrointestinal origin arriving in the ninth and tenth cranial nerves. This information is also relayed to higher levels of the neural axis including hypothalamic magnocellular neurosensory neurones. In the rat, the excitability of SON magnocellular neurosecretory neurones is clearly influenced by stimuli arising

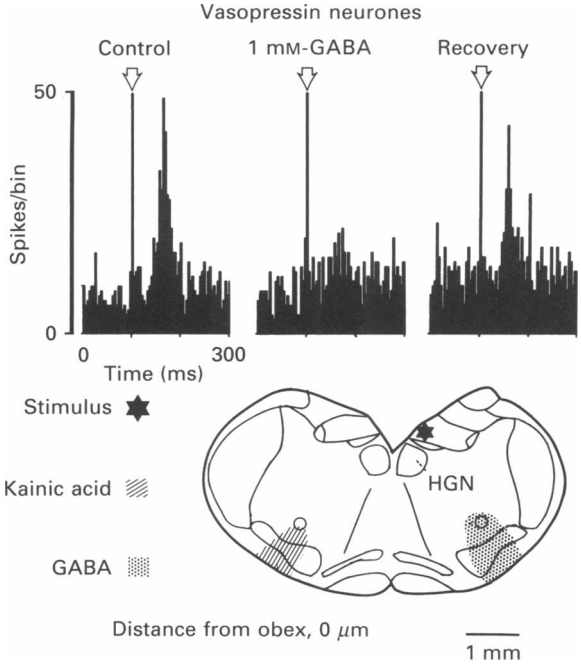

Fig. 6. The abbreviated peristimulus histograms represent the responses of eight out of eleven vasopressin neurones. The control response (after a kainic acid lesion in the contralateral ventrolateral medulla) illustrating an increase in excitability to the caudal nucleus tractus solitarii stimulus undergoes marked but reversible attentuation during microinjections (volume < 0·01 μl) of 1 mM-GABA in the ipsilateral ventrolateral medulla. HGN, hypoglossal nucleus.

from diverse peripheral sources, including sensory receptors in the nipple (Lincoln & Wakerley, 1975) and kidney (Day & Ciriello, 1987), carotid baroreceptors and chemoreceptors (Harris, 1979), hepatic portal osmoreceptors (Baertschi & Vallet, 1981), and gastric mechano- and chemoreceptors (Renaud *et al.* 1987). However, the organization of the ascending pathways and neurotransmitters involved in the transfer of this information centrally beyond the CNTS is not well defined and still under investigation (see Kannan, Yagi & Sawaki, 1981; Banks & Harris, 1984; Harris, Ferguson & Banks, 1984; Nosaka, 1984; Blessing & Willoughby, 1985*a*,*b*; Day & Sibbold, 1988). The present study provides further details on connections of CNTS with the SON, and its influence on the excitability of vasopressin and oxytocin neurones.

From a methodological viewpoint, an anatomical study of SON afferents using retrograde tracers must take into account the irregular dimensions (approximately, 300 μM in diameter, 1500 μm in length) and location of the nucleus. The ventral

transpharyngeal approach to the hypothalamus utilized here has two advantages over the dorsal stereotaxic approach: improved accuracy by direct positioning to the tip of a micropipette within the nucleus and lack of injury to the medial forebrain bundle which contains axons of passage ascending from medullary neurones. In view of the small dimensions of the nucleus, a non-diffusable tracer such as rhodamine-tagged latex microspheres is deemed superior because they remain confined to the injection site (cf. Katz, Burkhalter & Dreyer, 1984), yet are readily pinocytosed by axon terminals in the SON and transported retrogradely in sufficient numbers within 18–24 h to permit clear visualization of afferent neurones located as distant as the caudal medulla (Plate 1*D*). Medullary cell counts (Table 1) for SON injections that are small and intermediate in size indicate that input from the CVLM is almost exclusively catecholaminergic and hence arises from the A_1 noradrenergic neurones (cf. Sawchenko & Swanson, 1981, 1982). Relative to the CVLM input, the ipsilateral CNTS innervation of SON involves only one-half to one-third of the comparable number of catecholaminergic neurones, and also has a minor contralateral component. The comparatively larger number of CVLM and CNTS neurones labelled by a SON injection that extends into the perinuclear zone (Table 1) suggests that axons of many of these medullary neurones terminate in the perinuclear region immediately dorsal to the SON. Neurones here can be labelled by small intranuclear injections (Fig. 1). The perinuclear zone therefore contributes to the local synaptic inputs to SON neurones, and also serves as a local integrative site for information ascending from CVLM and CNTS catecholaminergic (and non-catecholaminergic) neurones.

Consideration of electrophysiological findings indicates that there are obvious functional differences between CVLM and CNTS inputs to magnocellular neurosecretory cells. Foremost is their response to electrical stimulation in CVLM which selectively excites only vasopressin neurones in both supraoptic (Day & Renaud, 1984) and paraventricular (PVN) nuclei (Day *et al.* 1984). This selectivity (for vasopressin neurones) agrees with the preferential anatomical distribution of catecholamine terminals in areas of SON and PVN that contain vasopressin immunoreactive magnocellular neurosecretory cells (McNeil & Sladek, 1980; Swanson *et al.* 1981; Sawchenko & Swanson, 1982; Hornsby & Piekut, 1987). The loss of CVLM-evoked responses following 6-hydroxydopamine pre-treatment (Day *et al.* 1984) combined with the ability for exogenously applied noradrenaline, in particular α_1-agonists, to activate SON vasopressin neurones *in vivo* (Day, Randle & Renaud, 1985) and *in vitro* (Armstrong, Gallagher & Sladek, 1986; Randle, Bourque & Renaud, 1986*a*; Yamashita, Inenaga & Kannan, 1987), and to promote the release of vasopressin (Armstrong *et al.* 1986; Randle, Mazurek, Kneifel, Dufresne & Renaud, 1986*c*; Willoughby, Jervois, Menadue & Blessing, 1987) suggests that CVLM stimulation excites vasopressin neurones through synaptic release of noradrenaline.

Although a precise physiological role of this prominent ascending CVLM innervation of hypothalamic vasopressin neurones remains to be determined, there are indications as to the type of information that is *not* transmitted through the CVLM. For instance, given the excitatory nature of the CVLM input to vasopressin neurones (Day *et al.* 1984; Day & Renaud, 1984), the depressant effects of peripheral baroreceptor activation on the firing of both vasopressin neurones (Jhamandas & Renaud, 1986) and A_1 neurones (McAllen & Blessing, 1987) suggests that trans-

mission of this type of cardiovascular information to higher centres is not relayed through CVLM. Failure to abolish baroreceptor-triggered inhibition of SON neurones by 6-hydroxydopamine lesions in VLM (Banks & Harris, 1984) or by the chemical lesions reported in the present study support this notion. Rather, lesion and stimulation data imply that relay of the inhibitory baroreceptor input to vasopressin neurones is through the dorsal pons and area of the locus coeruleus (Kannan *et al.* 1981; Banks & Harris, 1984). On the other hand, CVLM A_1 neurones are more likely to be activated by unloading of baroreceptors consequent to hypovolaemic and non-hypovolaemic haemorrhage and carotid occlusion, potent stimuli for vasopressin release (Share & Levy, 1966; Clark & Roche e Silva, 1967; Arnauld, Czernichow, Fumoux & Vincent, 1977). Single cell data in the rabbit reveal that a population of A_1 neurones actually increases its activity under these conditions (McAllen & Blessing, 1987). Interference with VLM function and its ascending noradrenergic projections prevents the anticipated rise in plasma vasopressin to these stimuli (Lightman *et al.* 1984; Blessing & Willoughby, 1985*b*), confirming its importance in the activation of hypothalamic vasopressin neurones.

Compared with the CVLM-evoked data, there is clearly an excitation of the majority of *both* vasopressin *and* oxytocin neurones in SON following electrical stimulation in CNTS (Table 2). Of particular note is the ability for chemical lesions and synaptic blockade in VLM to selectively interrupt the CNTS-evoked responses of vasopressin neurones. Such manipulations did not alter the CNTS-evoked responses of oxytocin neurones. We therefore conclude that the CNTS projects directly to oxytocin neurones whereas any CNTS influence on vasopressin neurones is relayed via the VLM. Since both catecholamine and non-catecholamine neurones in CNTS participate in the SON input, transmitters other than catecholamines may participate in the electrically evoked responses of oxytocin neurones. For example, there is now immunocytochemical evidence for two neuropeptides i.e. somatostatin-28 (Sawchenko, Benoit & Brown, 1988) and β-inhibin (Sawchenko, Plotsky, Pfeiffer, Cunningham, Vaughan, Rivier & Vale, 1988) in CNTS neurones that project to SON.

Still open to question is the nature of the visceral information transferred to SON from CNTS (or for that matter CVLM) neurones. There is growing evidence in favour of a parcelling of afferent inputs to the NTS (Kalia, Fuxe, Hökfelt, Johansson, Lang, Ganten, Cuello & Terenius, 1985). In the rat, for example, baroreceptors would thus be predominently represented rostrally and chemoreceptors caudally (Spyer, 1981; Housley, Martin-Body, Dawson & Sinclair, 1987) although this has not yet been verified by means of electrical recordings from CNTS neurones. According to recent electrophysiological studies, the A_2 catecholamine neurones in the CNTS lack a somatic sensory input and do not receive a significant baroreceptor input, but are powerfully excited by vagal stimulation (Moore & Guyenet, 1983, 1985). Hence, in the rat the information travelling in the noradrenergic portion of the CNTS – SON pathway may be gastrointestinal rather than cardiovascular in origin. This may be a reflection of this species; in the cat, NTS neurones projecting to paraventricular and supraoptic nuclei are apparently excited by electrical stimulation of carotid sinus and aortic depressor nerves (Ciriello & Caverson, 1984). Nevertheless, gastric distention is a potent excitant of NTS neurones in the rat (Raybould, Gayton & Dockray, 1985; Banks & Harris, 1987) as is the peripheral administration of CCK-8

(Raybould *et al.* 1985). Since both stimuli selectively excite SON oxytocin neurones (Renaud *et al.* 1987) there exists the strong possibility that this is one form of visceral information that could be directly transmitted to oxytocin neurones by a direct input from CNTS neurones.

In summary, the present anatomical data indicate that the SON receives bilateral input from both CVLM and CNTS. The CVLM innervation is the more prominent and is predominantly catecholaminergic. The CNTS input arises from both catecholamine and non-catecholamine neurones. Whereas electrophysiological data indicate that the CVLM input is selectively excitatory to vasopressin neurones, stimulation in the CNTS excites a majority of both oxytocin and vasopressin neurones. The CNTS-evoked activation of vasopressin neurones appears to be relayed via the A_1 neurones in the CVLM, while the input to oxytocin neurones is evidently direct. The nature of the visceral information transmitted by these two inputs to SON and the association of specific visceral signals with catecholaminergic *versus* non-catecholaminergic CNTS neurones projecting to the SON may be more readily defined in future investigations should it be possible to selectively alter function in any one of these pathways.

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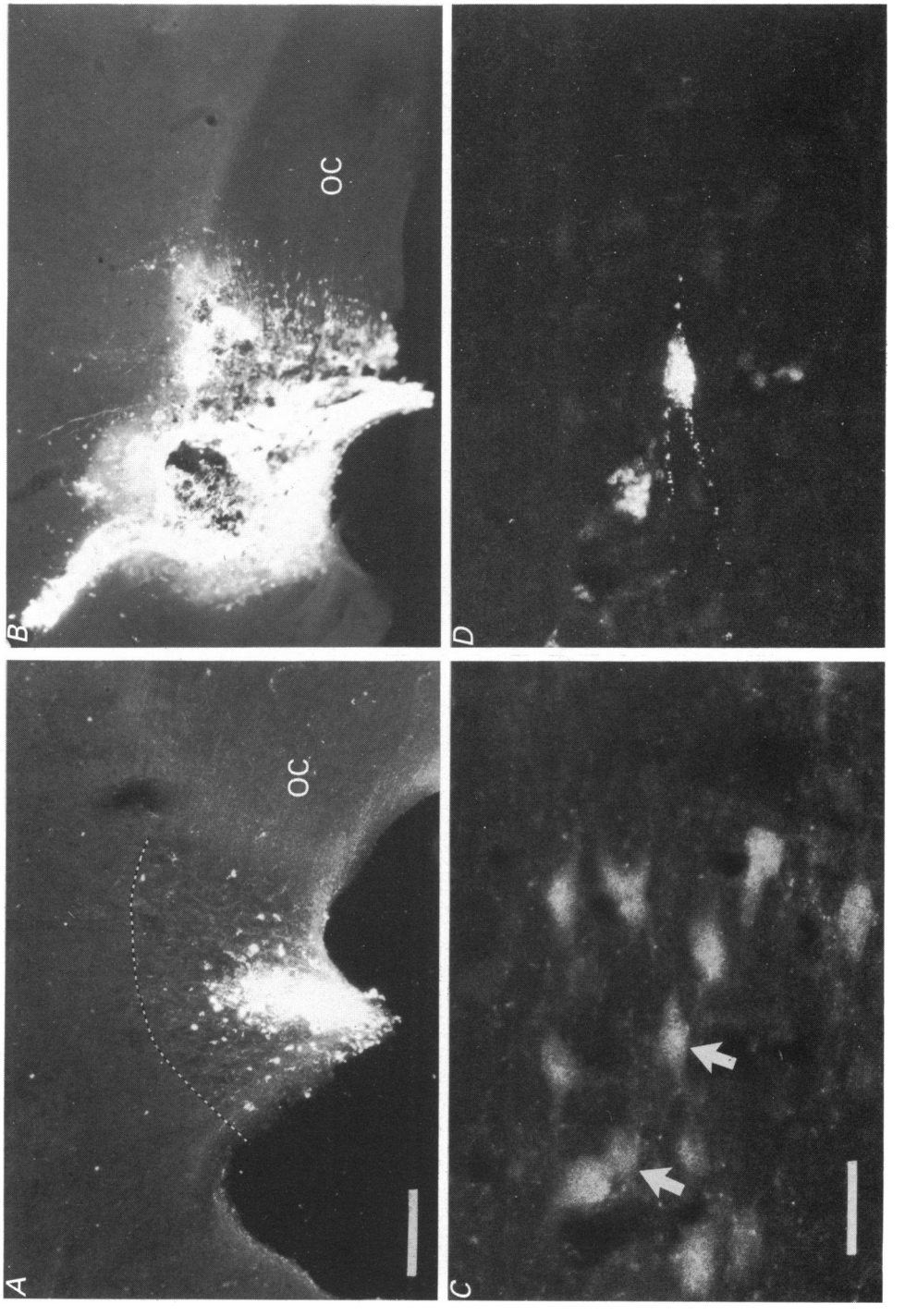
EXPLANATION OF PLATE

PLATE 1

A and *B*, photomicrographs illustrate small and large injections of rhodamine microspheres in the supraoptic nucleus (SON). In *A* the limits of the SON are traced by a dashed line to emphasize that the injection is solely within the nucleus. In *B* the injection clearly extends beyond the boundary of the SON. *C* and *D*, photographs of the same coronal section through the caudal nucleus tractus solitarii following a small latex-bead injection in the ipsilateral SON. Of the catecholamine fluorescent A_2 neurones illustrated in *C* there are two neurones (arrows) that also contain rhodamine microspheres, shown in *D*. Scale bars: 100 μm in *A* and *B*, 30 μm in *C* and *D*.



(*Facing p.* 294)