A γ-AMINOBUTYRIC-ACID-MEDIATED BARORECEPTOR INPUT TO SUPRAOPTIC VASOPRESSIN NEURONES IN THE RAT

By JACK H. JHAMANDAS and LEO P. RENAUD

From the Neurosciences Unit, Montreal General Hospital and McGill University, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4

SUMMARY

1. Extracellular recordings in pentobarbitone anaesthetized male Long–Evans rats examined the influence of electrical stimulation in the diagonal band of Broca on the excitability of 113 putative vasopressin-secreting and 22 putative oxytocin-secreting neurosecretory neurones in the hypothalamic supraoptic nucleus.

2. Single pulse or repetitive (5–20 Hz) stimulation in the ventral part of the diagonal band evoked a prominent reduction in the excitability of 83 % of vasopressin-secreting neurones with no effect on the remainder. Amongst oxytocin-secreting neurones, 59 % were unresponsive, 27 % responded with an increase in activity while only 14 % revealed an inhibitory pattern similar to vasopressin-secreting neurones.

3. Diagonal band stimulation-evoked inhibitions were reversibly abolished by local pressure applications of bicuculline methiodide (100 μM) to twenty out of twenty vasopressin-secreting cells tested, whereas strychnine sulphate (100 μM) was without effect on four out of four cells tested.

4. In five out of five vasopressin-secreting cells tested, bicuculline applications reversibly abolished the reduction in their activity that follows peripheral baroreceptor activation. Failure to alter baroreflex-evoked depressions in firing during similar trials with prazosin hydrochloride (10 μM, six cells tested), timolol maleate (20 μM, six cells tested) or strychnine sulphate (100 μM, three cells tested) indicated the specificity of bicuculline's action.

5. These findings suggest that a GABAergic pathway from the diagonal band of Broca preferentially innervates vasopressin-secreting neurosecretory supraoptic nucleus (s.o.n.) neurones, and support the view that the baroreflex-induced depression in firing of s.o.n. vasopressin-secreting neurones is mediated in large part through this input.

INTRODUCTION

The magnocellular vasopressin (VP) and oxytocin (OXY)-synthesizing neurones of the rat hypothalamic supraoptic nucleus (s.o.n.) project almost exclusively to the neurohypophysis (Sherlock, Field & Raisman, 1975) where hormone is released in proportion to their level of excitability (cf. Poulain & Wakerley, 1982 for review). Recent electrophysiological studies have focused attention on known afferents to s.o.n. in order to assess their role in regulating the excitability of neurosecretory

neurones. It appears that VP- and OXY-secreting neurones both receive excitatory inputs from the subfornical organ (Sgro, Ferguson & Renaud, 1984) and inhibitory connexions from amygdala and lateral septum (Poulain, Ellendorf & Vincent, 1980; Cirino & Renaud, 1985). In contrast, a more selective innervation pattern is revealed by electrical stimulation of the A1 noradrenergic cell group which excites only VP-secreting neurones (Day & Renaud, 1984). A similar selectivity of excitatory or inhibitory inputs to VP-secreting neurones follows activation of carotid chemo-receptors and peripheral baroreceptors respectively (cf. Harris, 1979) and is now used to differentiate between putative OXY- and VP-secreting cells during *in vivo* electro-physiological studies (cf. Day, Ferguson & Renaud, 1984).

Previous reports as to the role of central noradrenergic structures on VP release are contradictory. It is generally considered that noradrenaline suppresses VP release through a β-adrenoreceptor-mediated mechanism, based largely on ionophoretic data (Barker, Crayton & Nicoll, 1971). While recent observations using pressure ejection techniques support a β-adrenoreceptor-mediated depressant action of nor-adrenaline when applied to s.o.n. neurones in millimolar concentrations, the excita-bility of these same neurones is enhanced by application of noradrenaline and α-agonists in micromolar concentrations (Day, Randle & Renaud, 1985). Moreover, activation of endogenous noradrenergic projections appears to excite VP-secreting neurones (see Renaud, Day, Randle & Bourque, 1985 for review). Our earlier observation that 6-hydroxydopamine-induced terminal catecholamine field depletion in s.o.n. had no effect on the depression in VP neuronal activity following baroreceptor activation (Day & Renaud, 1984) suggested that this response was mediated by another transmitter, possibly γ-aminobutyric acid (GABA). The present study was further prompted by reports of immunoreactive GABAergic neurones in the diagonal band of Broca (Nagai, McGeer & McGeer, 1983; Panula, Revuelta, Cheney, Wu & Costa, 1984). In this area, we have noted enhanced firing of s.o.n. projecting neurones during baroreceptor activation (Jhamandas & Renaud, 1986) coincidental with the anticipated reduction in firing of VP-secreting neurones. We now report (a) that electrical stimulation in the diagonal band of Broca depresses the firing of s.o.n. VP neurones preferentially and (b) that the GABA antagonist bicuculline blocks both diagonal band- and peripheral baroreceptor-evoked reductions in their excitability. A portion of these data was reported briefly (Jhamandas & Renaud, 1985).

METHODS

Surgical preparation

Male Long–Evans rats (150–250 g) were anaesthetized initially with intraperitoneal sodium pentobarbitone (50 mg/kg) and maintained with supplemental doses of 2–4 mg given into the external jugular every 1–3 h. The femoral artery and vein were catheterized so as to record blood pressure and administer a peripheral vasoconstrictor (metaraminol, 2–10 µg) respectively. Heart rate was monitored continuously and body temperature was maintained at 37 °C. Following tracheal intubation the ventral surface of the hypothalamus was surgically exposed using the transpharyngeal approach.

Electrophysiology

Neurosecretory cells in the s.o.n. were identified by antidromic activation following stimulation (pulse duration 0·05 ms, intensity < 1 mA) of their axon terminals in the neurohypophysis.

Extracellular recordings were obtained through micropipettes (10–20 MΩ impedance) filled with
3·0 M-potassium acetate, amplified conventionally, bandpass filtered, displayed on an oscilloscope
and led through a window discriminator to an on-line PDP 11/23 computer programmed for spike
train analysis.

Cathodal pulses (0·05 ms duration, 100–400 μA) were delivered to the area of the diagonal band
of Broca through a monopolar, glass-insulated tungsten electrode (tip exposure 75–100 μm, base
diameter 25–50 μm) mounted on a micromanipulator and inserted through the ventral exposure.
The anode was attached to the exposed jaw muscles. The stimulation current was verified by
measuring the voltage drop across a 100 kΩ resistor placed in series with the stimulating electrode.

Classification of s.o.n. neurosecretory cells

In the rat, magnocellular neurosecretory cells may exhibit irregular, continuous or phasic firing
(Poulain & Wakerley, 1982). As reported previously (Day *et al.* 1984), their further classification
into putative VP-secreting or OXY-secreting cells depends on their response to activation of
peripheral baroreceptors, achieved by raising arterial blood pressure with a brief intravenous
infusion of metaraminol. Units demonstrating phasic or continuous activity that was transiently
suppressed by this manoeuvre were classified as VP secreting; those whose continuous firing was
unresponsive to baroreceptor activation were classified as OXY secreting (cf. Harris, 1979). Most
cells were located in the posterior and ventral portions of the s.o.n., where a majority of the
neurosecretory neurones are VP immunoreactive (cf. Sawchenko & Swanson, 1982).

Pharmacology

Drugs (from Sigma, St. Louis, MO, U.S.A.) applied to single neurones by pressure ejection (cf.
Day *et al.* 1985) included bicuculline methiodide (BMI, 100 μM), strychnine sulphate (100 μM),
prazosin hydrochloride (10 μM) and timolol maleate (20 μM). The concentrations chosen were
sufficient to block the responses of s.o.n. neurones to their appropriate agonists, i.e. γ-aminobutyrate,
glycine and noradrenaline in micromolar or millimolar concentrations respectively. Each drug was
dissolved in 0·9 % (w/v) NaCl at the time of experiment; prazosin was dissolved in dimethyl-
sulphoxide prior to dilution in saline. Drugs were ejected by a pressure device (Picospritzer, General
Valve Corp., NJ, U.S.A.) using 1–20 lb/in² applied to single channels of a 3-barrel micropipette
whose tip (total diameter 2·5–10 μm) was set back from that of the recording electrode by 30–50 μm.
Possible pressure-related effects were assessed by careful monitoring of spike amplitude; whenever
spike amplitude changed abruptly consequent to the application of a pressure pulse, the data were
excluded from further data analysis. Drug concentrations refer to the intrabarrel composition; one
would expect considerable dilution immediately upon ejection from the micropipette tip.

Histology

At the conclusion of each experiment the location of the stimulating electrode tip in the diagonal
band of Broca was marked by a small anodal lesion (200 μA d.c., 10 s). Animals were deeply
anaesthetized and perfused through the heart with saline followed by 10 % formaldehyde.
Stimulation sites were identified in 50 μm serial sections of the brain cut with a vibratome and
stained with thionin.

RESULTS

Diagonal band of Broca stimulation

Data from 135 antidromically identified and spontaneously active neurosecretory
neurones included 113 classified as putative VP neurones and 22 classified as putative
OXY neurones. Diagonal band of Broca stimulation differentially influenced the
excitability of cells in each group.

Among the putative VP cell population, stimulation sites confined to the ventral
half of the diagonal band of Broca (Fig. 1) altered the excitability of 83 % of neurones,
while the remainder were unresponsive. Responses consisted of a short latency
(mean 8·6±0·9 ms, s.e. of mean) reduction in excitability (Fig. 2*A*) lasting up to
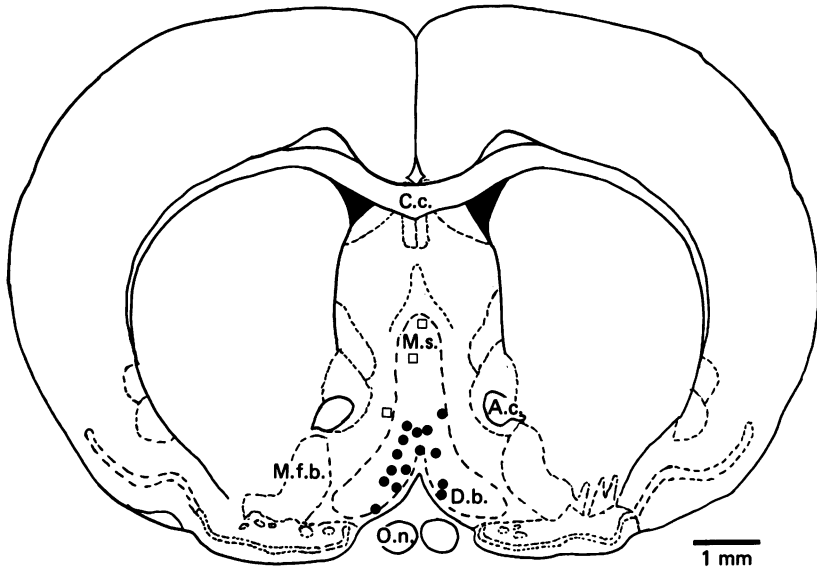100 ms (mean 49·0±3·2 ms) which could be evoked by currents of 100 μA or less.

Fig. 1. Symbols superimposed on a schematic coronal section of the rat brain depict the location of the electrode tips in the diagonal band of Broca (d.b.) and median septum (m.s.) where stimulation evoked a reduction in the excitability of s.o.n. VP neurones (●) or was without effect (□). Abbreviations: o.n., optic nerve; m.f.b., median forebrain bundle; a.c., anterior commissure; c.c., corpus callosum.

Responses of larger magnitude resulted from graded increments in either intensity or number of stimuli (Fig. 3 *A* and *B*). More prolonged changes in excitability could be achieved by trains of stimuli. As illustrated in Fig. 4, prolonged repetitive stimuli applied at frequencies above 2 Hz delayed or totally prevented spontaneous firing. At higher frequencies, e.g. 20 Hz, brief bursts of stimulation could prematurely terminate an ongoing spontaneous discharge (Fig. 5) mimicking the neuronal response that can be achieved by a brief increase in arterial pressure (cf. Fig. 7). These stimulations did not effect any detectable change in systemic blood pressure. The relationship of stimulus frequency to efficacy of response was not analysed in detail.

These observations contrasted sharply with data obtained from putative OXY neurones where the majority (59 %) were unaffected by stimulation with identical (or higher) magnitudes at the same locations. Only 14 % of OXY neurones displayed responses that resembled those seen in VP cells while the remaining 27 % demonstrated a prolonged (range 80–200 ms; mean $147 \cdot 0 \pm 16 \cdot 8$ ms) increase in excitability at latencies between 15 and 60 ms (mean $41 \cdot 0 \pm 7 \cdot 4$ ms) (Fig. 2*B*). On no occasion was the nature of an OXY-cell response changed by alteration in stimulus intensity.

For comparison, ten unidentified (i.e. non-neurosecretory) neurones located in the immediate vicinity of the s.o.n. were also tested. Six of these cells displayed an increase in excitability, with either long or short (Fig. 2*C*) duration responses.

Effects of bicuculline on stimulus-evoked inhibition

GABA is viewed as a major central inhibitory neurotransmitter candidate (Krnjevic, 1974). There is biochemical (Tappaz, Brownstein & Kopin, 1977; Meyer,
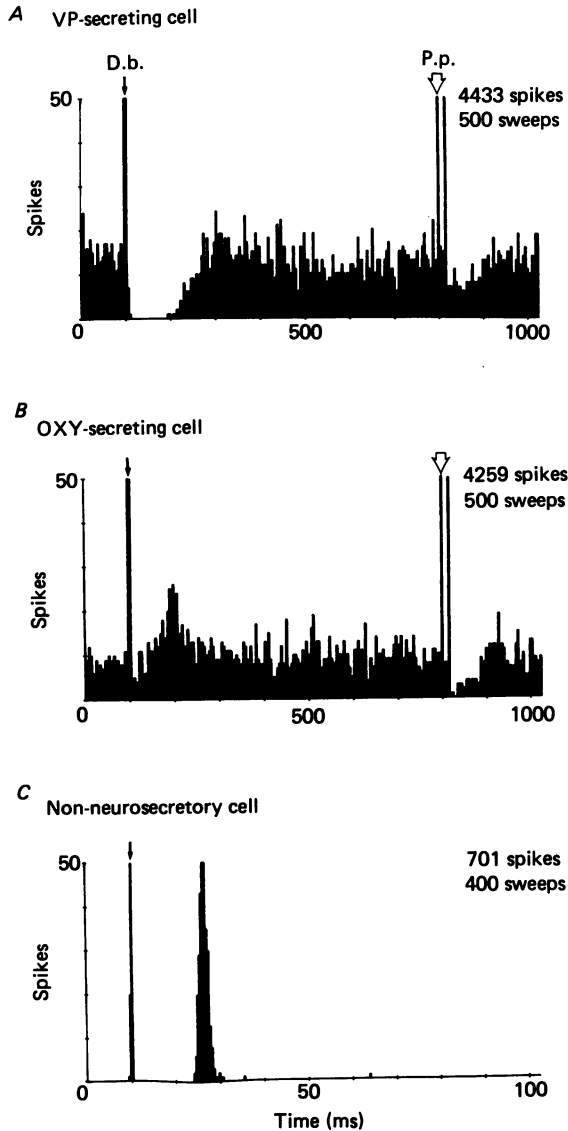
Fig. 2. Peri-stimulus histograms display the contrasting responses of s.o.n. neurones to single shock stimulation in the diagonal band (d.b., filled arrow) and posterior pituitary (p.p., open arrow). The antidromic spike produces the sharp response after the pituitary stimulus, set at 1·5 times threshold. In *A*, note the prominent reduction in excitability after d.b. stimulation. In *B*, data from an OXY neurone illustrate a long duration, low probability increase in excitability following d.b. stimulation. In *C*, data from a non-neurosecretory neurone illustrate one of the varied patterns of excitatory responses that could be observed. Resolution 4·0 ms per bin in *A* and *B*, 0·4 ms per bin in *C*; stimulus intensity 100 μA throughout.
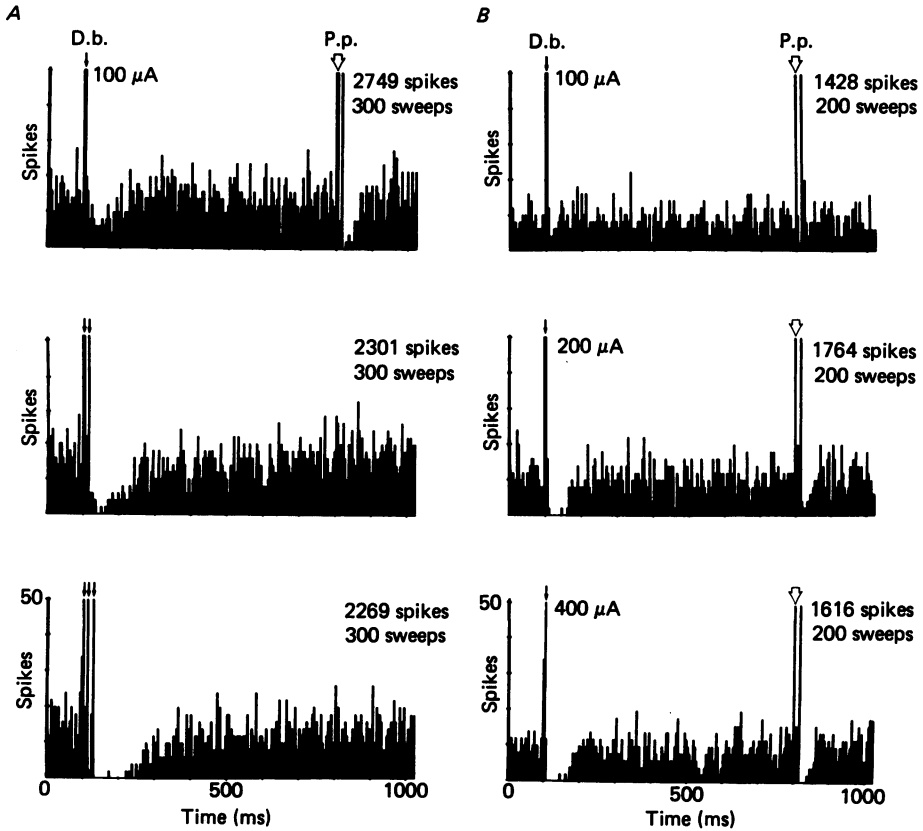
Fig. 3. *A*, peri-stimulus histograms from a VP neurone illustrate a progressive increase in the magnitude of response with increasing numbers of stimuli (100 μA, 10 ms apart). *B*, data from the same cell show the effects of graded increments (100–400 μA) in diagonal band (d.b.) stimulus intensity, and 1·1, 1·3 and 1·5 times threshold for posterior pituitary (p.p.) antidromic activation respectively.

Oertel & Brownstein, 1980) and immunohistochemical (Perez de la Mora, Possani, Tapia, Teran, Palacios, Fuxe, Hökfelt & Ljungdahl, 1981; van den Pol, 1985) evidence of GABA in the s.o.n. area and its application by ionophoresis depresses the excitability of s.o.n. cells (Bioulac, Gaffori, Harris & Vincent, 1978; Arnauld, Cirino, Layton & Renaud, 1983). GABA's contribution to the suppressant effects of diagonal band of Broca stimulation on VP neurones was evaluated by examining whether the evoked response pattern could be altered through local application of the GABA antagonist bicuculline. In twenty out of twenty cells tested, bicuculline reversibly abolished the stimulus-evoked inhibition and in six cells, unmasked a late onset, long duration increase in excitability (Fig. 6). The latter was not investigated further.

Strychnine, a glycine antagonist, may also alter GABA-mediated responses when applied in higher concentrations (Davidoff, Aprison & Werman, 1969). Strychnine was without any effect on synaptic inhibition in four out of four cells tested.
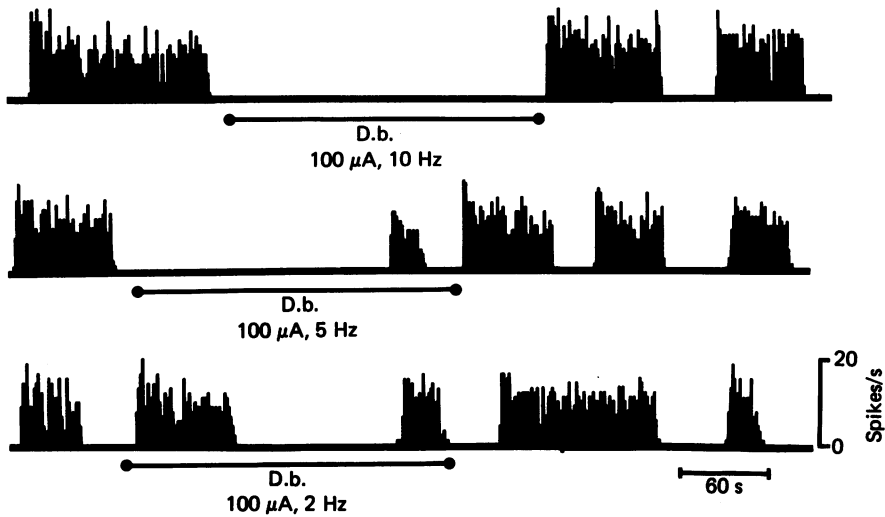
Fig. 4. Rate-meter records from a phasically firing VP neurone illustrate the reduction in spontaneous activity that can be achieved by repetitive diagonal band (d.b.) stimulation at frequencies above 2 Hz. Each bin represents 1·0 s.
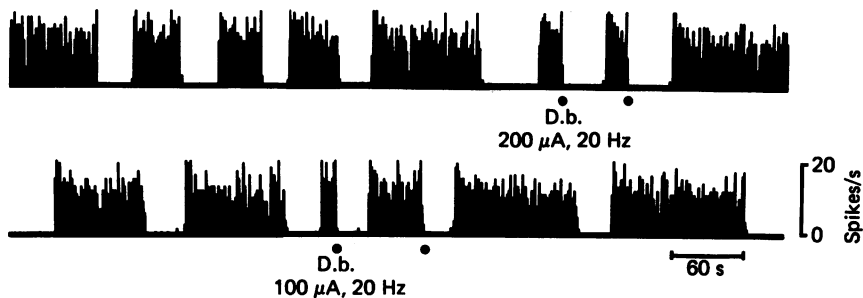

Fig. 5. Rate-meter records from the same cell as in Fig. 4 demonstrate that cessation of spontaneous firing can be evoked by brief bursts of stimuli (twenty pulses at each point) applied to diagonal band (d.b.) at 20 Hz.

Bicuculline and baroreceptor-induced inhibition

The ability of bicuculline to alter the suppression in firing of VP neurones consequent to a brief rise in arterial pressure was also examined. In five out of five cells tested, bicuculline applied concomitantly with activation of peripheral arterial baroreceptors reversibly suppressed their anticipated reduction in firing (Fig. 7). Failure to obtain similar results with application of prazosin (six out of six cells), timolol (six out of six cells) and strychnine (three out of three cells) attested to the selectivity of bicuculline's blockade of the evoked response.
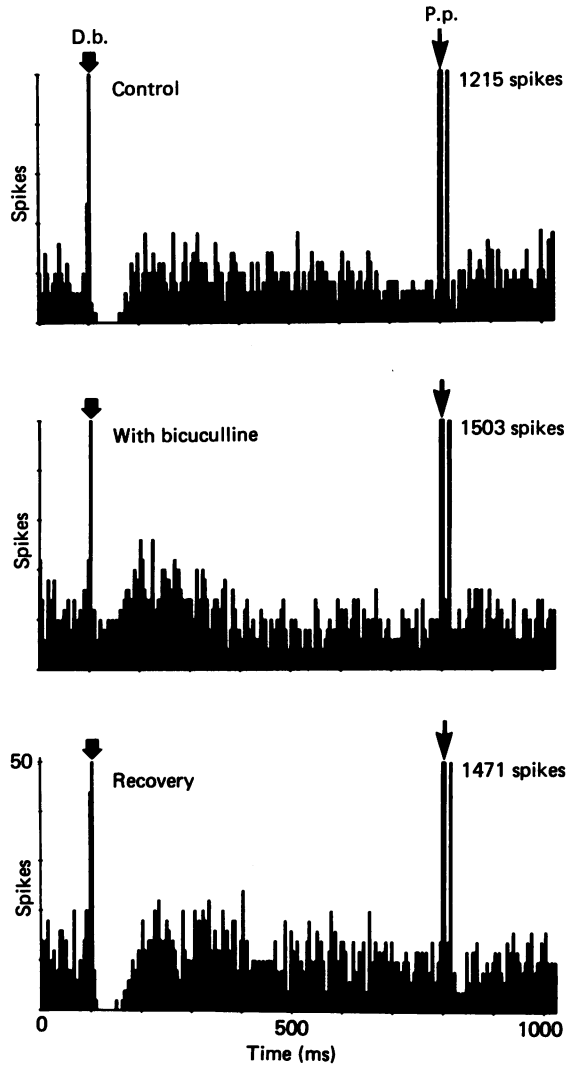
Fig. 6. Peristimulus histograms from a VP neurone illustrate control responses to diagonal band (d.b.) stimulation (top), abolition of d.b. responses in the presence of bicuculline methiodide (100 μM, middle row) and recovery of response (lowest records). Note the low probability, long duration (250 ms) increase in excitability during bicuculline application. Recovery is achieved within 4 min. Posterior pituitary (p.p.) stimulus intensity 1·1 times threshold throughout.

DISCUSSION

The results indicate that electrical stimulation of the diagonal band of Broca activates an inhibitory projection to s.o.n. that is directed principally to the VP cell population. It is possible that these effects are secondary to the activation of descending fibres of passage arising in other structures where electrical stimulation can depress the excitability of s.o.n. neurones, notably the lateral septum (Poulain *et al.* 1980; Cirino & Renaud, 1985) and nucleus acumbens (Shibuki, 1984). However,
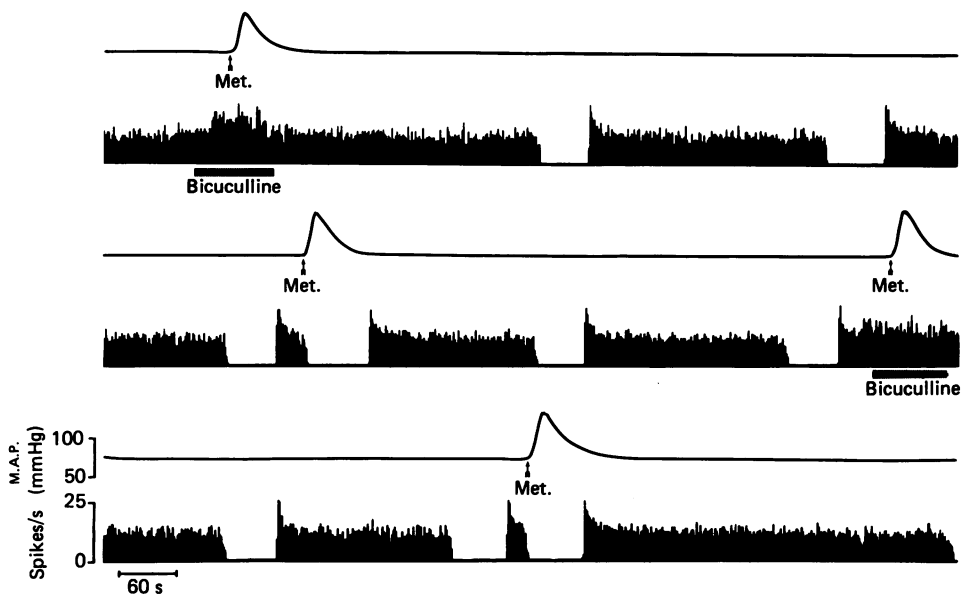
Fig. 7. Continuous rate-meter records (lower trace) from a phasically firing VP neurone demonstrates a typical reduction in firing induced by a rise in mean arterial pressure (M.A.P. upper traces) consequent to intravenous administration of metaraminol (Met. arrow). Locally applied bicuculline methiodide (100 μM) by pressure ejection (horizontal line) reversibly abolishes the metaraminol-induced response.

the data from the studies cited fail to indicate the observed selectivity for VP neurones. A more likely explanation is that our stimuli activated a pathway that arises in the diagonal band. Anatomical evidence of a projection from diagonal band to the area of the s.o.n. is available (Zaborszky, Leranth, Makara & Palkovits, 1975; Tribollet, Armstrong, Dubois-Dauphin & Dreifuss, 1985) and we have recently reported antidromic activation of diagonal band neurones after electrical stimulation of the s.o.n. (Jhamandas & Renaud, 1986). Moreover, intracellular recordings in s.o.n. neurones maintained *in vitro* in perfused hypothalamic explants that exclude septum and nucleus accumbens reveal that electrical stimulation of the diagonal band evokes prominent post-synaptic inhibitory potentials (i.p.s.p.s) in a majority of ventrally located (i.e. probable VP-synthesizing) s.o.n. neurones (Randle, Bourque & Renaud, 1985). Thus, the pathway from diagonal band is either direct to s.o.n. neurones or is mediated by local inhibitory interneurones (see below).

The ability for locally applied bicuculline to alter both diagonal band- and baroreflex-evoked inhibition of firing of s.o.n. VP neurones identifies GABA as a probable neurotransmitter mediating both responses. This is supported by *in vitro* intracellular recordings which reveal that diagonal band-evoked i.p.s.p.s and conductance changes are chloride dependent, bicuculline sensitive and mimicked by exogenously applied GABA and $GABA_A$ agonist muscimol (Randle *et al.* 1985; Randle, Day, Jhamandas, Bourque & Renaud, 1986a). Indeed GABA's presence in s.o.n. has been clearly determined on both biochemical (Tappaz *et al.* 1977; Meyer *et al.* 1980) and anatomical grounds (Perez de la Mora *et al.* 1981; Vincent, Hökfelt

& Wu, 1982; Tappaz, Wassef, Oertel, Paut & Pujol, 1983; van den Pol, 1985). While immunohistochemical studies indicate that GABA-containing fibres can account for its presence within the s.o.n. itself, the distribution of GABAergic cells extends forward from the perinuclear area into and beyond the diagonal band (Nagai *et al.* 1983; Panula *et al.* 1984). We have recently reported (Jhamandas & Renaud, 1986) that diagonal band neurones projecting to s.o.n. increase their firing during metaraminol-induced hypertension, coinciding with the suppression in firing of s.o.n. VP neurones. We now postulate that diagonal band neurones with this activity profile are GABAergic interneurones mediating a central inhibition of VP-magnocellular neurones in response to peripheral baroreceptor activation. Double-labelling neuroanatomical tracer studies can be expected to address this issue more directly.

This hypothesis is contradictory to a widely held belief that central catecholamines, notably noradrenaline, depress VP secretion by a tonic inhibitory action on VP-secreting neurones (e.g. Armstrong, Sladek & Sladek, 1982; Blessing, Sved & Reis, 1982). However, recent evidence no longer supports this view (Renaud *et al.* 1985; Willoughby & Blessing, 1985; Armstrong, Gallagher & Sladek, 1986). Although lesions of the locus coeruleus interfere with baroreflex-induced depressions of s.o.n. neurones (Banks & Harris, 1984), it would appear that this noradrenergic cell group does not constitute the final pathway whereby peripheral baroreceptor information reaches VP neurones. Depletion of the terminal catecholamine plexus in s.o.n. or paraventricular nucleus by 6-hydroxydopamine does not alter the depressant response of putative VP neurones to a brief metaraminol-induced systemic hypertension (Day & Renaud, 1984), but does obliterate excitatory responses evoked in s.o.n. VP neurones by electrical stimulation of the A1 cell group, the principal site of origin in catecholamine fibres in s.o.n. (Sawchenko & Swanson, 1982). In addition, exogenously applied noradrenaline in micromolar concentrations excited VP neurones *in vivo* (Day *et al.* 1985). Confirmatory *in vitro* studies reveal both enhanced neuronal excitability and bursting activity patterns (Randle, Bourque & Renaud, 1984), membrane depolarization (Randle *et al.* 1986*a*) and enhanced release of VP (Randle, Mazurek, Kneifel, Dufresne & Renaud, 1986*b*), mediated through the activation of an α_1-receptor. Thus, the available data overwhelmingly favours the proposal that A1 (and possibly A2) noradrenergic neurones directly excite VP-secreting s.o.n. cells. This would be ideally suited from central transmission of information arising in peripheral chemoreceptors (cf. Harris, 1979) or hepatic portal osmoreceptors (Baertschi & Vallet, 1981). While the locus coeruleus does appear to participate in central pathways transmitting data on peripheral baroreceptor activity, the precise mechanisms and central pathways whereby these inputs ascend to the proposed GABAergic neurones in the rostral hypothalamus require more detailed evaluation.

REFERENCES

ARMSTRONG, W. E., GALLAGHER, M. J. & SLADEK, C. D. (1986). Noradrenergic stimulation of supraoptic neuronal activity and vasopressin release *in vitro*: mediation by an α_1-receptor. *Brain Research* **365**, 192–197.

ARMSTRONG, W. E., SLADEK, C. D. & SLADEK, J. R. (1982). Characterization of noradrenergic control of vasopressin release by the organ-cultured rat hypothalamo-neurohypophyseal system. *Endocrinology* **111**, 273–279.

ARNAULD, E., CIRINO, M., LAYTON, B. S. & RENAUD, L. P. (1983). Contrasting actions of amino acids, acetylcholine, noradrenaline and leucine enkephalin on the excitability of supraoptic vasopressin-secreting neurons. *Neuroendocrinology* **36**, 187–196.

BAERTSCHI, A. J. & VALLET, P. G. (1981). Osmosensitivity of the hepatic portal vein area and vasopressin release in rats. *Journal of Physiology* **315**, 217–230.

BANKS, D. & HARRIS, M. C. (1984). Lesions of the locus coeruleus abolish baroreceptor-induced depression of supraoptic neurones in the rat. *Journal of Physiology* **355**, 383–398.

BARKER, J. L., CRAYTON, J. W. & NICOLL, R. A. (1971). Noradrenaline and acetylcholine responses of supraoptic neurosecretory cells. *Journal of Physiology* **218**, 19–32.

BIOULAC, B., GAFFORI, O., HARRIS, M. & VINCENT, J.-D. (1978). Effects of acetylcholine, sodium glutamate and GABA on the discharge of supraoptic neurons in the rat. *Brain Research* **154**, 159–162.

BLESSING, W. W., SVED, A. F. & REIS, D. J. (1982). Destruction of noradrenergic neurons in rabbit brainstem elevates plasma vasopressin, causing hypertension. *Science* **217**, 661–663.

CIRINO, M. & RENAUD, L. P. (1985). Influence of lateral septum and amygdala stimulation on the excitability of hypothalamic supraoptic neurons. An electrophysiological study in the rat. *Brain Research* **326**, 357–361.

DAVIDOFF, R. A., APRISON, M. H. & WERMAN, R. (1969). The effects of strychnine on the inhibition of interneurons by glycine and γ-aminobutyric acid. *International Journal of Neuropharmacology* **8**, 191–194.

DAY, T. A., FERGUSON, A. V. & RENAUD, L. P. (1984). Facilitatory influences of noradrenergic afferents on the excitability of rat paraventricular nucleus neurosecretory cells. *Journal of Physiology* **355**, 237–249.

DAY, T. A., RANDLE, J. C. R. & RENAUD, L. P. (1985). Opposing α- and β-adrenergic mechanisms mediate dose dependent actions of noradrenaline on supraoptic vasopressin neurons *in vivo*. *Brain Research* **358**, 171–179.

DAY, T. A. & RENAUD, L. P. (1984). Electrophysiological evidence that noradrenergic afferents selectively facilitate the activity of supraoptic vasopressin neurons. *Brain Research* **303**, 233–240.

HARRIS, M. C. (1979). Effects of chemoreceptor and baroreceptor stimulation on the discharge of hypothalamic supraoptic neurones in rats. *Journal of Endocrinology* **82**, 115–125.

JHAMANDAS, J. H. & RENAUD, L. P. (1985). Influence of anteroventral third ventricle (AV3V) stimulation on the excitability of hypothalamic supraoptic neurosecretory neurons. *Neuroscience Abstracts* **11**, 1235.

JHAMANDAS, J. H. & RENAUD, L. P. (1986). Diagonal band neurons may mediate arterial baroreceptor input to hypothalamic vasopressin-secreting neurons. *Neuroscience Letters* **65**, 214–218.

KRNJEVIK, K. (1974). Chemical nature of synaptic transmission in vertebrates. *Physiological Reviews* **54**, 418–540.

MEYER, D. K., OERTEL, W. H. & BROWNSTEIN, M. J. (1980). Deafferentiation studies on the glutamic acid decarboxylase content of the supraoptic nucleus of the rat. *Brain Research* **200**, 165–168.

NAGAI, T., McGEER, P. L. & McGEER, E. G. (1983). Distribution of GABA-T intensive neurons in the rat forebrain and midbrain. *Journal of Comparative Neurology* **218**, 220–238.

PANULA, P., REVUELTA, A. V., CHENEY, D. L., WU, J.-Y. & COSTA, E. (1984). An immunohistochemical study on the location of GABAergic neurons in rat septum. *Journal of Comparative Neurology* **222**, 69–80.

PEREZ DE LA MORA, M., POSSANI, L. D., TAPIA, R., TERAN, L., PALACIOS, R., FUXE, K., HÖKFELT, T. & LJUNGDAHL, A. (1981). Demonstration of central γ-aminobutyric-containing nerve terminals by means of antibodies against glutamate decarboxylase. *Neuroscience* **6**, 875–895.

POULAIN, D. A., ELLENDORF, F. & VINCENT, J. D. (1980). Septal connections with identified oxytocin and vasopressin neurones in the supraoptic nucleus of the rat. An electrophysiological investigation. *Neuroscience* **5**, 379–387.

POULAIN, D. A. & WAKERLEY, J. B. (1982). Electrophysiology of hypothalamic magnocellular neurones secreting oxytocin and vasopressin. *Neuroscience* **7**, 773–808.

RANDLE, J. C. R., BOURQUE, C. W. & RENAUD, L. P. (1984). α-adrenergic activation of rat hypothalamic supraoptic neurons maintained *in vitro*. *Brain Research* **307**, 374–378.

RANDLE, J. C. R., BOURQUE, C. W. & RENAUD, L. P. (1985). Spontaneous and evoked IPSPs in rat supraoptic nucleus neurosecretory neurons *in vitro*. *Proceedings of the Canadian Federation of Biological Societies* **28**, 93.

RANDLE, J. C. R., DAY, T. A., JHAMANDAS, J., BOURQUE, C. W. & RENAUD, L. P. (1986a). Neuropharmacology of supraoptic nucleus neurons: noradrenergic and GABAergic receptors. *Federation Proceedings* (in the Press).

RANDLE, J. C. R., MAZUREK, M., KNEIFEL, D., DUFRESNE, J. & RENAUD, L. P. (1986b). Alpha-1 adrenergic receptor activation releases vasopressin and oxytocin from perfused hypothalamic explants. *Neuroscience Letters* **65**, 219–233.

RENAUD, L. P., DAY, T. A., RANDLE, J. C. R. & BOURQUE, C. W. (1985). *In-vivo* and *in-vitro* electrophysiological evidence that central noradrenergic pathways enhance the activity of hypothalamic vasopressinergic neurosecretory cells. In *Vasopressin*, ed. SCHRIER, R. W., pp. 385–393. New York: Raven Press.

SAWCHENKO, P. E. & SWANSON, L. W. (1982). The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. *Brain Research Reviews* **4**, 275–325.

SGRO, S., FERGUSON, A. V. & RENAUD, L. P. (1984). Subfornical organ-supraoptic nucleus connections: an electrophysiological study in the rat. *Brain Research* **303**, 7–13.

SHERLOCK, D. A., FIELD, P. A. & RAISMAN, P. G. (1975). Retrograde transport of horseradish peroxidase in the magnocellular neurosecretory system of the rat. *Brain Research* **88**, 403–414.

SHIBUKI, K. (1984). Supraoptic neurosecretory cells: synaptic inputs from the nucleus accumbens in the rat. *Experimental Brain Research* **53**, 341–348.

TAPPAZ, M. L., BROWNSTEIN, M. J. & KOPIN, I. J. (1977). Glutamate decarboxylase (GAD) and γ-aminobutyric acid (GABA) in discrete nuclei of hypothalamus and substantia nigra. *Brain Research* **125**, 109–121.

TAPPAZ, M. L., WASSEF, M., OERTEL, W. H., PAUT, L. & PUJOL, J. F. (1983). Light- and electron-microscopic immunocytochemistry of glutamic acid decarboxylase (GAD) in the basal hypothalamus: morphological evidence for neuroendocrine gamma-aminobutyrate (GABA). *Neuroscience* **9**, 271–287.

TRIBOLLET, E., ARMSTRONG, W. E., DUBOIS-DAUPHIN, M. & DREIFUSS, J. J. (1985). Extrahypothalamic afferent inputs to the supraoptic nucleus area of the rat as determined by retrograde and anterograde tracing techniques. *Neuroscience* **15**, 135–138.

VAN DEN POL, A. (1985). Dual ultrastructural localization of two neurotransmitter-related antigens: colloidal gold-labeled neurophysin-immunoreactive supraoptic neurons receive peroxidase-labeled glutamate decarboxylase- or gold-labeled GABA-immunoreactive synapses. *Journal of Neuroscience* **5**, 2940–2954.

VINCENT, S. R., HÖKFELT, T. & WU, J.-Y. (1982). GABA neuron systems in hypothalamus and the pituitary gland. *Neuroendocrinology* **34**, 117–125.

WILLOUGHBY, J. O. & BLESSING, W. W. (1985). Neuropeptide Y and endogenous noradrenaline stimulate secretion of vasopressin in the rat. *Neuroscience Abstracts* **11**, 1057.

ZABORSZKY, L., LERANTH, C., MAKARA, G. B. & PALKOVITS, M. (1975). Quantitative studies on the supraoptic nucleus in the rat. II. Afferent fiber connections. *Experimental Brain Research* **22**, 525–540.