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# LOCAL INHIBITORY SYNAPTIC INPUTS TO NEURONES OF THE PARAVENTRICULAR NUCLEUS IN SLICES OF RAT HYPOTHALAMUS

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SUMMARY

1. Intracellular recordings were obtained from neurones in the region of the paraventricular nucleus in slices of rat hypothalamus. Glutamate microdrops were applied to the surface of the slices at sites dorsal, lateral and ventral to the paraventricular nucleus to selectively activate local presynaptic neurones. The $\gamma$-aminobutyric $acid_A$ ($GABA_A$)-receptor antagonists picrotoxin or bicuculline were bath-applied to block synaptic inhibition.

2. Glutamate microapplication caused a tonic depolarization and often repetitive action potentials in twenty of forty-seven recorded cells. This was probably caused by the direct exposure of the dendrites of the recorded cells to the glutamate microdrops.

3. Glutamate microstimulation elicited inhibitory synaptic responses in nine of forty-seven neurones tested. Glutamate microdrops caused discrete, hyperpolarizing postsynaptic potentials (PSPs) in four cells recorded with microelectrodes containing potassium acetate and evoked depolarizing PSPs in four cells recorded with KCl-filled microelectrodes. Glutamate microapplication inhibited spontaneous spike firing in another cell recorded with a potassium acetate microelectrode.

4. Bath application of $GABA_A$-receptor antagonists completely blocked the hyperpolarizing PSPs elicited by glutamate microstimulation in three of three cells recorded with potassium acetate electrodes and the depolarizing PSPs in two of two cells recorded with KCl electrodes, indicating they were inhibitory PSPs caused by the release of GABA. Suppression of $GABA_A$-mediated synaptic inhibition did not reveal any glutamate-evoked excitatory PSPs.

5. Recorded cells were identified as magnocellular, parvocellular or non-paraventricular bursting neurones on the basis of their electrophysiological properties. Direct depolarization and local inhibitory synaptic responses were observed in all three cell types.

6. Several conclusions can be drawn from these data: (1) functional glutamate

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receptors are distributed throughout neuronal populations in the paraventricular region of the hypothalamus, confirming and extending previous observations; (2) local synaptic inputs to neurones in the paraventricular nucleus are primarily inhibitory, supplied by perinuclear GABAergic neurones; (3) both magnocellular and parvocellular subpopulations receive local inhibitory synaptic inputs. The possibility that these local GABAergic circuits mediate inhibitory inputs to paraventricular neurones from limbic structures is discussed.

## INTRODUCTION

The paraventricular nucleus of the hypothalamus is a complex structure comprised of several different populations of cells divided into two main groups, the magnocellular and the parvocellular neurones. The magnocellular neurones are neuroendocrine cells which, together with the magnocellular neurones of the supraoptic nucleus, project to the neurohypophysis and secrete oxytocin or vasopressin. The parvocellular neurones consist of two distinct cell populations, the neurosecretory neurones and the preautonomic neurones. The parvocellular neurosecretory cells project to the median eminence and release hypophysiotrophic factors, which regulate hormone secretion from the anterior pituitary. The preautonomic neurones are not neurosecretory, but project caudally to autonomic centres in the brainstem and spinal cord (Armstrong, Warach, Hatton & McNeill, 1980; Swanson & Kuypers, 1980; Sawchenko & Swanson, 1982).

The magnocellular and parvocellular neurones of the paraventricular nucleus have been found to have different electrophysiological properties such that often they can be identified from intracellular recordings without the need to perform subsequent anatomical analyses (Tasker & Dudek, 1991; Hoffman, Tasker & Dudek, 1991). Magnocellular neurones of the paraventricular nucleus have electrical properties very similar to magnocellular neurones of the supraoptic nucleus, including evidence of a prominent A current and linear current–voltage relations; parvocellular neurones as a group differ from magnocellular neurones by their capacity to generate small low-threshold $Ca^{2+}$ spikes. A third population of neurones with distinct electrical properties has been identified just outside the paraventricular nucleus. These cells generate large low-threshold $Ca^{2+}$ spikes and bursts of action potentials and sometimes display repetitive bursting behaviour (Poulain & Carette, 1987; Tasker & Dudek, 1991). Their close proximity to paraventricular magnocellular neurones and the observation that they sometimes project into the lateral magnocellular region of the paraventricular nucleus has led to the speculation that these bursting cells may provide local synaptic inputs to paraventricular neurones (Poulain & Carette, 1987).

The evidence from several areas of the nervous system (e.g. the retina, the ventral horn of the spinal cord and the CA3 region of the hippocampus) suggests that local synaptic circuits are an important element of the synaptic organization governing neuronal output (see Shepherd, 1990). Evidence for local synaptic regulation of hypothalamic neurones has come primarily from anatomical and indirect physiological studies. According to these reports, the paraventricular nucleus receives projections from several different hypothalamic areas, including the preoptic area, the ventromedial nucleus, the anterior hypothalamus, the lateral hypothalamus, the

suprachiasmatic nucleus and the arcuate nucleus (see Swanson & Sawchenko, 1983). Connections between the bilateral supraoptic and paraventricular nuclei are suggested by the observation that oxytocin or acetylcholine applied to cells of one nucleus *in vivo* facilitates the firing of oxytocinergic neurones in the contralateral nucleus (Moos & Richard, 1989). The electron microscopic finding that oxytocin-containing synaptic terminals contact synaptically with oxytocinergic neurones in the supraoptic nucleus provides anatomic support for these connections (Theodosis, 1985). Inhibitory postsynaptic potentials (IPSPs) evoked in supraoptic magnocellular neurones by electrical stimulation of the diagonal band of Broca have been attributed to local inhibitory interneurones (Randle, Bourque & Renaud, 1986), but the possibility that they were mediated by projections from extrinsic neurones cannot be excluded. Thus data are available which suggest the existence of local synaptic inputs to hypothalamic neurones, but local synaptic circuits have yet to be demonstrated conclusively.

Electrical stimulation is the most commonly used method for studying synaptic circuitry. This technique evokes action potentials in local cell bodies and dendrites, as well as in axons projecting into the region of stimulation from extrinsic sources. It therefore cannot be used to selectively study synaptic interactions between specific populations of neurones without potential contamination from axons of passage. The techniques of simultaneous intracellular and extracellular or paired intracellular recordings from two cells are the most definitive means of determining synaptic connectivity. However, these are technically the most problematic procedures due to the difficulty of obtaining two recordings simultaneously and the need to record from large numbers of neurones in order to overcome the inherent sampling bias. Furthermore, dual recordings are the least likely to provide useful data when local synaptic connections are sparse, since negative data in this case have limited value. Both with extracellular recordings *in vivo* (Goodchild, Dampney & Bandler, 1982) and with intracellular recordings *in vitro* (Christian & Dudek, 1988), glutamate has been shown to evoke action potentials only when applied to neuronal cell bodies and dendrites, and not when applied to axons of passage. Glutamate microapplication, therefore, provides a means by which selective stimulation of specific populations of local neurones can be achieved. By recording postsynaptic events in a neurone in response to focal glutamate microstimulation, one can determine whether the recorded cell receives synaptic inputs from neurones located in the region of glutamate application. This technique effectively reduces the sampling bias encountered with dual recordings, since glutamate microdrops activate groups of cells rather than single neurones.

We have performed experiments using intracellular recordings and glutamate-microdrop application in hypothalamic slices to study local synaptic circuits in the paraventricular nucleus. We have found physiological evidence for glutamate receptors and for local inhibitory synaptic circuits among neurones in the region of the paraventricular nucleus. A preliminary report of these findings has appeared (Tasker & Dudek, 1988).

## METHODS

*Slice preparation*

Adult Sprague–Dawley rats (150–250 g, $n = 41$) were decapitated and their brains removed and immersed in cold (1–2 °C), oxygenated artificial cerebrospinal fluid (ACSF) for 1 min. One or two coronal hypothalamic slices, 400–500 $\mu$m in thickness, were cut just caudal to the optic chiasm with a vibroslice tissue slicer (Campden Instruments) and placed in a ramp-style interface recording chamber. The ACSF contained (mM): 124 NaCl, 3 KCl, 2·4 $CaCl_2$, 26 $NaHCO_3$, 1·3 $MgSO_4$, 1·24 $NaH_2PO_4$ and 11 glucose. The concentrations of $CaCl_2$ and of $MgSO_4$ were raised to 4 mM in some experiments in which KCl-filled recording electrodes were used in order to reduce spontaneous synaptic activity. ACSF was heated to 32–34 °C and pumped into the recording chamber where it was drawn up over the slices with threads of gauze. A gas mixture of 95% $O_2$ and 5% $CO_2$ was humidified and directed over the surface of the slices. Slices were allowed to equilibrate in the recording chamber for approximately 2 h prior to the start of experiments.

*Electrophysiological techniques*

Recording electrodes were pulled from glass capillaries (1·0 mm o.d., 0·5 mm i.d., American Glass Co.) on a Flaming-Brown puller (Sutter Instruments) and filled with 4 M potassium acetate or 2 M KCl. Electrode resistances ranged from 75 to 150 MΩ. Cell impalement was achieved by advancing electrodes through the slice in 4 $\mu$m steps with a piezoelectric microdrive (Nanostepper, Adams List) and oscillating the negative capacitance feedback. Electrical signals were recorded using an intracellular amplifier with a bridge circuit (Neurodata Instruments or Axon Instruments), stored on videotape and plotted on a laser printer or played out directly on a pen recorder.

*Glutamate microapplication*

Glutamate microapplication was used to stimulate selectively the somata and dendrites of local hypothalamic neurones without activating axons projecting to the paraventricular nucleus from extrinsic sources. The purpose of glutamate microapplication, therefore, was to evoke a synaptic response in recorded cells by directly activating presynaptic neurones situated just outside the paraventricular nucleus. Glutamate (10–100 mM in ACSF) was micro-applied by pressure (18–36 N) to the surface of the slice with a single- or double-barrelled micropipette (5–10 $\mu$m i.d.). Glutamate microdrops measured approximately 100–250 $\mu$m in diameter when applied on a flat surface (e.g. on a microscope slide). Cells were tested for synaptic responses to glutamate at depolarized and hyperpolarized membrane potentials to increase the driving force for inhibitory and excitatory synaptic currents, respectively. When cells did not respond to glutamate microapplication or when glutamate microdrops caused a direct depolarization of the recorded cell, the glutamate micropipette was repositioned until a seemingly pure synaptic response was elicited. For each recorded cell, glutamate was first applied at a site lateral to the paraventricular nucleus. The glutamate micropipette was then moved and glutamate reapplied usually at one or more positions ventral and/or dorsal to the paraventricular nucleus. In some experiments, ACSF was applied through the second barrel of the pipette in the same location and with the same pressure pulses as the glutamate to control for mechanical artifacts of microdrop application.

*Drug application*

Picrotoxin (50 $\mu$M) or bicuculline (10 $\mu$M) was added to the perfusate to block $GABA_A$-receptor-mediated inhibition. $GABA_A$ antagonists were applied to block glutamate-evoked IPSPs as well as to reveal local excitatory synaptic circuits that might otherwise be undetectable due to presynaptic inhibition or postsynaptic shunting of synaptic currents.

## RESULTS

A total of forty-seven neurones in the region of the hypothalamic paraventricular nucleus were recorded intracellularly and tested for local synaptic inputs with glutamate microstimulation. Recorded cells had a mean resting membrane potential of $62·1 \pm 2·1$ mV (S.E.M., $n = 16$), input resistance of $247 \pm 15$ MΩ ($n = 22$) and action potential amplitude of $61·6 \pm 1·5$ mV, measured from threshold to peak ($n = 22$).

*Direct activation by glutamate*

In twenty of the cells tested, glutamate microapplication in one or more sites elicited a tonic depolarization which often caused repetitive spike firing and spike inactivation (Fig. 1). Depolarization was presumably caused by diffusion of the

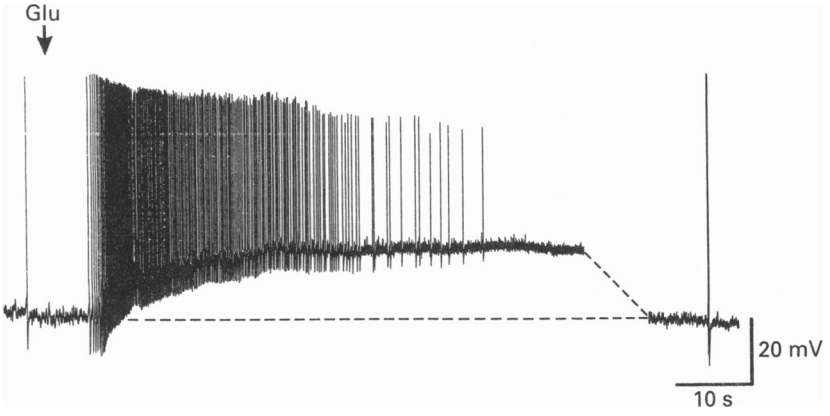


Fig. 1. Direct activation of a paraventricular neurone by glutamate microapplication. Glutamate microdrops (Glu) caused depolarization and repetitive action potentials, suggesting the glutamate was acting directly on the recorded cell. Gradual inactivation of action potential discharge occurred with maintained depolarization. The membrane potential returned to resting level (dashed line) and spontaneous action potentials were seen again after several tens of seconds (break in trace). This cell was a putative parvocellular neurone.

glutamate microdrops such that the glutamate came into direct contact with the dendrites of the recorded cells. In some cases, the recorded cell did not recover from the robust excitation by glutamate. In experiments in which the recorded cell did recover, the glutamate pipette was moved to one or more different positions (see Methods) and the application repeated in order to determine if a synaptic response could be evoked at another site.

*Glutamate-evoked synaptic responses*

Glutamate microdrops were usually applied at two or more positions around the paraventricular nucleus and, whenever possible, recorded cells were tested for synaptic responses at several different membrane potentials (see Methods). Glutamate microapplication lateral or ventrolateral to the paraventricular nucleus elicited a distinct inhibitory synaptic response in nine of forty-seven cells. In cells recorded with potassium acetate electrodes, glutamate microdrops caused repetitive, hyperpolarizing postsynaptic potentials (PSPs) in four cells (Fig. 2). The latency-to-onset of the PSPs was 0·1 to 3·2 s and the duration was 1·4 to 42 s; the duration of the train of PSPs varied with the size of the glutamate drop, larger drops eliciting responses of longer duration. Bath application of picrotoxin (50 $\mu$M) or bicuculline (10 $\mu$M) blocked the hyperpolarizing PSPs in three of three neurones tested (Fig. 3),

indicating that they were IPSPs generated by the release of GABA and mediated by $GABA_A$ receptors. The glutamate-evoked IPSPs summated to cause a tonic hyperpolarization in one of these cells (Fig. 4*A*); this cell was one of the three in which the IPSPs were blocked by bicuculline (Fig. 4*B*). Glutamate microapplication

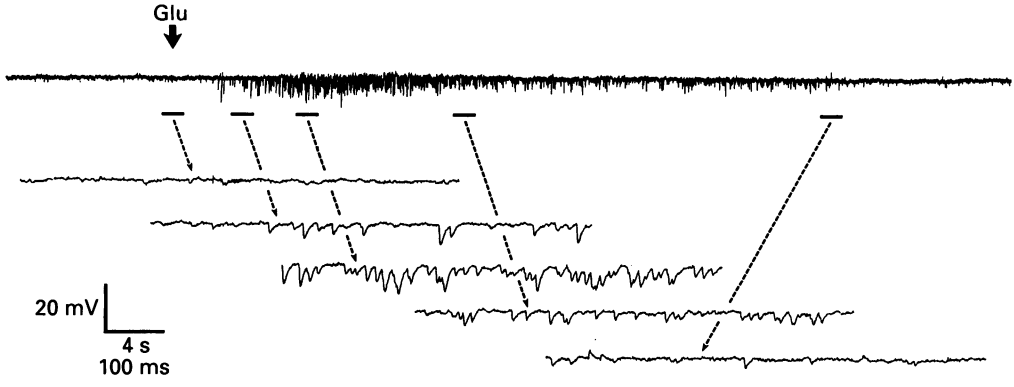


Fig. 2. IPSPs elicited in a cell in the paraventricular nucleus by glutamate microstimulation of local presynaptic neurones. The cell was depolarized approximately 10 mV with continuous positive current to increase the driving force of the $Cl^-$ current; very little spontaneous synaptic activity was seen. Glutamate microdrops applied ventrolaterally to the paraventricular nucleus (Glu) elicited a robust burst of IPSPs which lasted for over 40 s (top trace). Segments of the top trace (bars) were expanded to show individual IPSPs (lower traces). Glutamate microapplication at sites lateral and dorsal to the paraventricular nucleus were less effective or ineffective, respectively, in eliciting a synaptic response. This cell showed electrical properties that were characteristic of magnocellular neurones, including phasic firing with tonic suprathreshold depolarization, and was located within the paraventricular nucleus. The upper time calibration pertains to the top trace and the lower calibration to the expanded traces.

in a fifth cell recorded with a potassium acetate electrode inhibited spontaneous spike firing. Of nine cells recorded with KCl electrodes, four responded to glutamate microdrops with repetitive, depolarizing PSPs. Bicuculline (10 $\mu$M) blocked the depolarizing PSPs in two of two cells tested, confirming that they were reversed IPSPs.

Clear EPSPs without tonic depolarization were not observed in response to glutamate microstimulation. Glutamate microdrops did cause an increase in EPSPs in three cells, but the EPSPs were accompanied or preceded by a depolarization. In one of these cells, summated EPSPs appeared to cause the membrane depolarization, but it was not possible to show conclusively that glutamate was not also acting directly on the cell membrane to cause depolarization. In a fourth cell, glutamate microdrops elicited an increase in PSPs, but it was not clear whether these events were EPSPs or IPSPs due to vigorous spontaneous synaptic activity. We were unable to determine whether the glutamate-evoked excitatory responses were purely synaptic in nature (and not also direct) and thus we cannot exclude the possibility that the glutamate microdrops were acting on the presynaptic terminals of projection neurones and not on the somata and dendrites of local presynaptic

neurones. Finally, blockade of synaptic inhibition with picrotoxin or bicuculline failed to reveal EPSPs in response to glutamate microstimulation in a total of seven cells tested.

*Cell identification*

Recorded cells were identified on the basis of electrophysiological criteria defined in previous reports (Tasker & Dudek, 1991; Hoffman *et al.* 1991). As described in

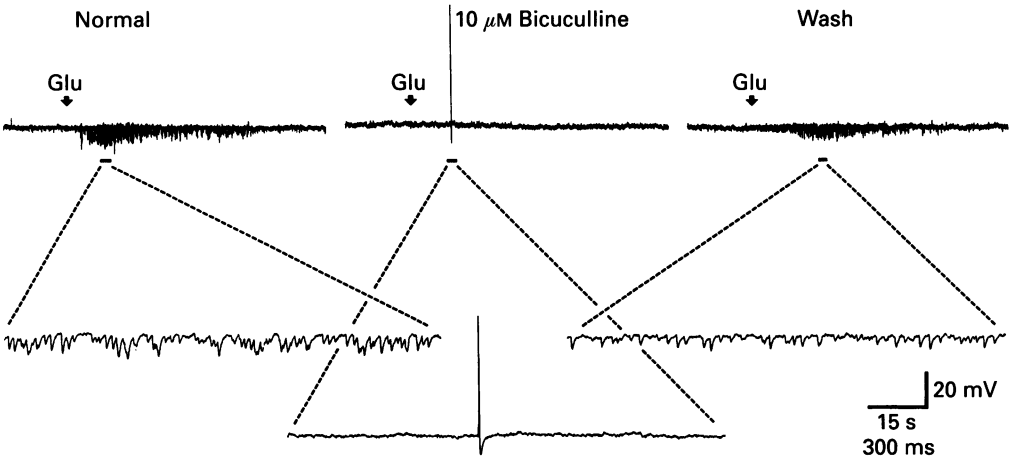


Fig. 3. Glutamate-evoked IPSPs were blocked by $GABA_A$-receptor antagonists. This recording is from the same putative magnocellular neurone as the recording shown in Fig. 2. The cell was depolarized approximately 10 mV with continuous current such that the membrane potential was just subthreshold for action-potential generation. The lower traces are expanded sweeps of the segments of the upper traces designated by the bars. Left traces, a glutamate microdrop applied in normal medium (Glu) evoked a burst of IPSPs. Middle traces, bath application of bicuculline (10 $\mu$M) blocked the synaptic response to an identical glutamate microdrop. The drop was applied in the same location and with the same application parameters as the microdrop applied in normal solution (i.e. the pipette was not moved and the pressure settings were not altered). There was no evidence for glutamate activation of local excitatory inputs, since EPSPs were not detected when inhibition was blocked. An action potential was generated from a single EPSP, which probably occurred spontaneously. Right traces, partial recovery of the inhibitory synaptic response to glutamate microstimulation was recorded after 1 h of wash-out of the bicuculline solution. The upper time calibration applies to the top traces and the lower calibration to the expanded traces.

those studies, the main electrical criterion used to identify the different cell populations was the capacity to generate low-threshold $Ca^{2+}$ spikes and the amplitude of the low-threshold $Ca^{2+}$ spikes (i.e. the capacity of the low-threshold spikes to generate bursts of fast action potentials); other distinguishing properties were current–voltage relations, evidence of a distinct A current and/or spontaneous bursting patterns (e.g. phasic firing). Thus, putative magnocellular neurones lacked low-threshold $Ca^{2+}$ spikes (LTS) and showed evidence of a prominent A current (non-LTS cells). Putative parvocellular neurones generated small low-threshold $Ca^{2+}$

spikes which did not elicit bursts of fast action potentials (non-bursting LTS cells). Non-paraventricular bursting neurones generated large low-threshold $Ca^{2+}$ spikes supporting bursts of fast action potentials and showed strong inward rectification (bursting LTS cells).

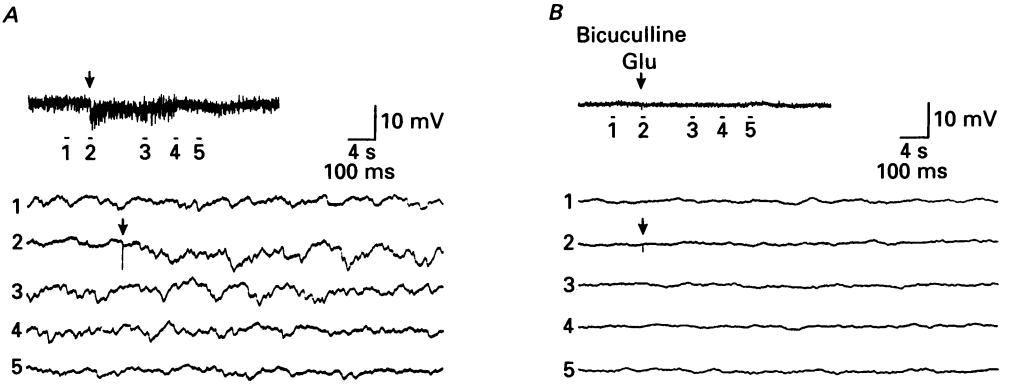


Fig. 4. Hyperpolarization evoked by glutamate microstimulation. This cell showed vigorous on-going synaptic activity. The lower traces labelled 1 to 5 in *A* and *B* are expansions of the segments in the upper traces designated by the bars with corresponding numbers. *A*, in normal ACSF, a glutamate microdrop applied lateral to the paraventricular nucleus elicited a hyperpolarization of the membrane potential. The hyperpolarization apparently was caused by the summation of multiple IPSPs, seen more clearly in the expanded traces below. *B*, bath application of bicuculline (10 $\mu$M) blocked not only the IPSPs evoked by the glutamate microdrop, but also the spontaneous IPSPs. No obvious glutamate-evoked EPSPs were detected when $GABA_A$-receptor-mediated inhibition was blocked. This cell had the electrical properties of a non-paraventricular bursting cell and was located dorsal to the paraventricular nucleus, suggesting it was neither a magnocellular nor a parvocellular neurone. The upper time calibrations apply to the top traces in *A* and *B* and the lower calibrations to the expanded sweeps below.

Cells of the three cell types were directly activated by glutamate microdrops. Four of the twenty cells depolarized by glutamate microapplication were putative magnocellular neurones. Ten of the cells were putative parvocellular neurones. One cell was identified as a non-paraventricular bursting neurone from its electrical properties. We did not identify five of the cells directly activated by glutamate microdrops.

Glutamate microstimulation did not evoke a synaptic response at all locations around the paraventricular nucleus. Glutamate microdrops were only effective in eliciting a synaptic response when applied lateral or ventrolateral to the nucleus (Fig. 5). Of the nine cells showing an inhibitory synaptic response to glutamate microstimulation, four were identified as putative magnocellular neurones, four as putative parvocellular neurones and one as a non-paraventricular bursting cell (based on their electrical properties). One of the putative magnocellular neurones generated a phasic firing pattern when depolarized with continuous positive current injection, suggesting that it may have been a vasopressinergic cell (Poulain & Wakerley, 1982).

## DISCUSSION

### *Glutamate microstimulation*

Intracellular recordings and glutamate microstimulation were used to study local synaptic interactions among neurones in the paraventricular region of the

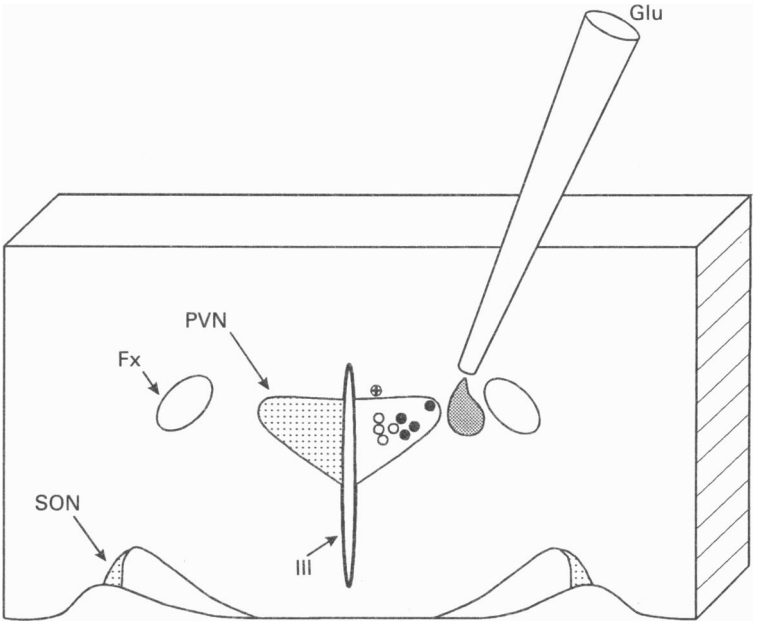


Fig. 5. Neurones responding synaptically to glutamate microstimulation. Glutamate (Glu) microdrops were usually applied in two or more positions around the paraventricular nucleus (PVN); IPSPs were elicited in positions lateral/ventrolateral to the paraventricular nucleus, as indicated by the glutamate drop. Approximate localization of cells responding synaptically to glutamate was achieved from visual placement of the recording electrode with respect to the paraventricular nucleus and landmarks such as the third ventricle (III) and the fornix (Fx). Responsive cells were identified as putative parvocellular (○), magnocellular (●) or non-paraventricular bursting cells (⊕) on the basis of their electrical properties. SON, supraoptic nucleus.

hypothalamus. Glutamate microapplication allowed us to stimulate hypothalamic cells selectively without activating axons of extrinsic neurones (Goodchild *et al.* 1982; Christian & Dudek, 1988). That PSPs in response to glutamate microstimulation were not mediated by receptors located on presynaptic terminals in our experiments (Forsythe & Clements, 1990) is suggested by the pure synaptic response (i.e. without direct depolarization) observed in nine of the recorded cells. Glutamate acting at receptors on presynaptic terminals would be expected to diffuse also to the receptors located on the postsynaptic membrane, which would cause direct depolarization in addition to synaptic activation of the postsynaptic cell. Synaptic activation with glutamate microstimulation was usually specific to the site of microdrop application

(i.e. glutamate microdrops were not effective in eliciting a synaptic response at all application sites around the paraventricular nucleus). Therefore, it should be possible in the future with this technique to map topographically the areas around (or inside) the paraventricular nucleus that contain cells presynaptic to paraventricular neurones.

*Direct activation with glutamate*

As in other structures of the central nervous system, glutamate is thought to be the main excitatory neurotransmitter in the hypothalamus. Hypothalamic neurones express genes which encode for glutamate receptors (Bettler *et al.* 1990). Iontophoretic application of glutamate activates cells in the supraoptic (Bioulac, Gaffori, Harris & Vincent, 1978; Arnaud, Cirino, Layton & Renaud, 1983) and paraventricular nuclei (Moss, Urban & Cross, 1972). Synaptic responses evoked by electrical stimulation of afferents to neurones in the supraoptic (Gribkoff & Dudek, 1990) and paraventricular nuclei (Wuarin & Dudek, 1991) are blocked by glutamate-receptor antagonists. In our experiments, glutamate microdrops caused tonic depolarization and spike generation in putative magnocellular and parvocellular neurones of the paraventricular nucleus and in non-paraventricular bursting cells. This response was presumably caused by glutamate diffusion into the nucleus (in the case of magnocellular and parvocellular neurones) where it acted directly on the dendrites or cell bodies of paraventricular neurones. These data confirm the previous evidence for glutamate receptors on paraventricular neurones and indicate that both magnocellular and parvocellular neurones, as well as non-paraventricular bursting neurones, have functional glutamate receptors. Our finding that glutamate activates local inhibitory neurones presynaptic to paraventricular neurones suggests that these perinuclear GABA-containing cells also have functional glutamate receptors. Whether these presynaptic inhibitory neurones and the non-paraventricular bursting neurones are the same cells remains to be determined.

*Glutamate-evoked synaptic inhibition*

Our results provide physiological evidence for inhibitory synaptic inputs to neurones in the region of the paraventricular nucleus from cells located outside the nucleus. Although the glutamate microdrops were applied outside the boundaries of the paraventricular nucleus on the basis of visual cues, we cannot rule out the possibility that the synaptic responses were mediated by local inputs from cells within the lateral paraventricular nucleus (due to diffusion of the glutamate microdrops into the nucleus). However, in a putative magnocellular neurone that was localized with biocytin injection and neurophysin immunohistochemistry on the lateral border of the nucleus in a separate study (see Fig. 2 of Hoffman *et al.* 1991), a pure synaptic response was evoked by glutamate microstimulation lateral to the recorded cell (i.e. probably outside the nucleus). The GABAergic cells that have been identified immunohistochemically within the paraventricular nucleus tend to be located in medial parvocellular regions (Meister, Hökfelt, Geffard & Oertel, 1988; Decavel & van den Pol, 1990) and it is unlikely that the glutamate microdrops diffused that far medially. Thus, projections from neurones located outside the paraventricular nucleus probably mediated these synaptic responses.

The inhibitory synaptic responses evoked by glutamate were blocked by $GABA_A$-receptor antagonists, suggesting they were mediated by GABA release. This local GABAergic projection to paraventricular cells probably originates in the population of GABA-containing cells that has been described in the area surrounding the paraventricular nucleus in immunohistochemical studies (Meister *et al.* 1988; Sakaue, Saito, Taniguchi, Baba & Tanaka, 1988). GABAergic synapses make up half of the total number of synapses impinging on hypothalamic neurones in the arcuate, supraoptic and paraventricular nuclei (Decavel & van den Pol, 1990), and GABAergic synapses account for most of the 'double' synapses (i.e. one presynaptic element contacting two postsynaptic elements in the same plane of section) which form selectively on supraoptic and paraventricular oxytocinergic cells during lactation (Theodosis, Paut & Tappaz, 1986; Theodosis & Poulain, 1989). Intracellular recordings in the hypothalamic explant have revealed robust inhibitory synaptic inputs to neurosecretory neurones of the supraoptic nucleus occurring spontaneously and in response to electrical stimulation of the diagonal band of Broca (Randle *et al.* 1986). Neurones in the paraventricular nucleus appear to receive an equally dense GABAergic innervation (Mugnaini & Oertel, 1985; Meister *et al.* 1988; Sakaue *et al.* 1988; Decavel, Dubourg, Leon-Henri, Geffard & Calas, 1989; Decavel & van den Pol, 1990) and it is likely that at least a proportion of this input derives from local sources. Indeed, recent anatomical findings from combined tract-tracing and immunohistochemical experiments suggest that a large proportion of the GABAergic inputs to the paraventricular nucleus arise locally (Roland, Brown & Sawchenko, 1991). Our physiological data corroborate these anatomical observations and further suggest that local GABAergic inputs to the paraventricular nucleus may be important for the regulation of both magnocellular and parvocellular neurones.

A relatively small percentage of the recorded cells (19%) showed an inhibitory synaptic response to glutamate microstimulation. Although this may indicate a paucity of inhibitory inputs to neurones of the paraventricular nucleus from perinuclear GABAergic neurones, it may also reflect technical limitations of glutamate microstimulation in the slice preparation. It is possible, for example, that many more paraventricular neurones receive local inhibitory inputs but that the presynaptic neurones are not contained within the slice or that the trajectory of their axons leaves the plane of the slice. Thus negative results cannot be interpreted necessarily as a lack of local synaptic inputs. However, from studies done in neocortical slices, it is clear that the density of local synaptic circuits in the hypothalamus is qualitatively much lower than that in the neocortex (Tasker, Peacock & Dudek, 1992).

*Glutamate-evoked synaptic excitation*

Although pure EPSPs (i.e. without direct membrane depolarization) were not seen in response to glutamate microstimulation, it is possible that local excitatory synaptic circuits are present in this region of the hypothalamus. Since glutamate-evoked depolarizations were accompanied sometimes by an apparent increase in EPSPs, some of these may have been due to the rapid summation of EPSPs or to the combination of a direct effect on the recorded cell and a simultaneous activation of presynaptic excitatory neurones. As discussed above, the failure to detect pure

EPSPs in response to glutamate microstimulation may have been caused by cutting the local excitatory projections in the slicing procedure (because of their trajectory out of the plane of the slice) or by the glutamate microdrops not reaching the excitatory presynaptic neurones when present in the slice. Nevertheless, the lack of a clear excitatory synaptic response to glutamate microapplication, both in normal solution and with inhibition blocked with GABA antagonists, suggests that local excitatory synaptic circuits are not abundant in this region of the hypothalamus.

*Possible functional significance*

Although it is premature to draw conclusions as to the physiological significance of a local inhibitory projection to paraventricular neurones, it is reasonable to speculate on the possible involvement of such a projection in neurosecretory function. Several limbic structures have been shown to exert an inhibitory influence on the electrical activity of supraoptic and paraventricular neurones recorded extracellularly *in vivo* (Pittman, Blume & Renaud, 1981; Ferreyra, Kannan & Koizumi, 1983). Stimulation of the septum inhibits the background firing of oxytocinergic neurones in the supraoptic and paraventricular nuclei and alters the periodicity of reflex milk ejections (Lebrun, Poulain & Theodosis, 1983). Autoradiographic and retrograde labelling studies have shown that projections from limbic structures such as the septum and subiculum do not enter the paraventricular nucleus but terminate in the area immediately surrounding it, forming a 'halo' around the nucleus (Sawchenko & Swanson, 1983; Silverman & Oldfield, 1984). The dendrites of paraventricular neurones tend to be restricted to the nucleus, with few dendritic branches extending outside the boundaries of the nucleus (van den Pol, 1982; but see Oldfield, Hou-Yu & Silverman, 1985). These results together suggest that the inhibitory influence limbic structures exercise on neurosecretory systems is transmitted via interneurones located around the paraventricular nucleus. Our findings indicate that there are local GABAergic neurones outside the paraventricular nucleus that provide inhibitory synaptic inputs to both magnocellular and parvocellular neurones, and suggest that the inhibitory influence of limbic structures on paraventricular neurosecretory neurones may be mediated by these perinuclear GABAergic interneurones.

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