RAT SUPRAOPTIC NEURONES: THE EFFECTS OF LOCALLY APPLIED HYPERTONIC SALINE

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SUMMARY

1. Extracellular action potentials were recorded from supraoptic neurones in lactating, urethane-anaesthetized rats. A microtap was used to apply a very small volume (about 10^{-7} ml.) of hypertonic saline (1–4 M-NaCl) to the immediate neighbourhood of these units over about 1 min.

2. Twenty-five of twenty-seven supraoptic neurones were excited by this local osmotic stimulus. The response of individual units was reversible and repeatable. Microtap applications of isotonic saline to supraoptic neurones were without observed effect.

3. Continuously firing supraoptic neurones responded to hypertonic saline with a smooth acceleration in firing rate. Phasic neurones showed an increase in the over-all level of activity, and in particular, a prolongation of the active phases. Slow, irregularly firing cells responded either with a smooth acceleration in firing rate, or with phasic behaviour.

4. The response to local hypertonic saline appears to be reasonably specific to the supraoptic nucleus. Of thirty-five neurones recorded close to the supraoptic nucleus but which were not antidromically activated from stimulation of the neural stalk, only nine responded to the local application of hypertonic saline.

5. Similarities between the manner of response of supraoptic neurones to local application of hypertonic saline and the manner of their response to systemic increases in the osmotic pressure of blood plasma support the hypothesis that supraoptic neurones are osmosensitive.

INTRODUCTION

It is widely accepted (Verney, 1947; Woods, Band & Bleier, 1966) that there are osmoreceptors close to the supraoptic nucleus. Vasopressinergic and oxytocinergic neurones in the supraoptic and paraventricular nuclei respond to a rise in plasma osmotic pressure with an over-all increase in activity, whether the rise is induced by dehydration (Walters & Hatton, 1974; Arnauld, Dufy & Vincent, 1975) or by intracarotid or I.P. injections of hypertonic saline (Dyball, 1971; Brimble & Dyball, 1977). However the neural origin of this response to osmotic stimuli has not been identified.

Recently, two groups of workers (Brimble, Haller & Wakerley, 1978; Hatton, Armstrong & Gregory, 1978) recorded from magnocellular neurosecretory cells *in*

vitro and obtained conflicting results. Both groups recorded unit activity from hypo-thalamic slices during changes in the osmotic pressure of the bathing medium. Brimble and co-workers observed no effects upon spontaneous or glutamate-induced activity in either the paraventricular nucleus or the supraoptic nucleus. Hatton and co-workers were unable to record from the supraoptic nucleus, but reported that some paraventricular neurones were excited by osmotic changes. Thus it remains controversial whether magnocellular neurosecretory cells are themselves osmo-receptors.

To establish that a particular neurone is an osmoreceptor it seems necessary to apply a local osmotic stimulus while recording the electrical activity of that neurone, and to compare the observed behaviour with the behaviour seen in response to systemic osmotic stimulation. In present experiments a microtap was used to apply very small amounts of hypertonic saline to the neighbourhood of antidromically identified single units in the rat supraoptic nucleus.

METHODS

Rats of 250–350 g from the A.R.C. Babraham Wistar colony were used at day 9–18 of lactation after overnight separation from their litters. Rats were anaesthetized with urethane (ethyl carbamate, I.P., 1·25 g/kg body wt.). One inguinal mammary gland was cannulated to monitor intramammary pressure. The trachea and a branch of the jugular were also cannulated. The hypothalamus was approached from the ventral surface (Dreifuss & Ruf, 1972). One small drill-hole exposed the neural stalk, a second aperture exposed the supraoptic nucleus unilaterally. A bipolar stainless steel stimulating electrode (SNEX 100, Rhodes Medical Instruments) was placed across the stalk. Heparinized saline (0·5–1 ml., I.V.) was injected to compensate for esti-mated blood loss. Rectal temperature was maintained at close to 37 °C. Plasma osmotic pressure was determined from 1 ml. blood samples using the freezing-point method with a Knauer Semimicro Osmometer.

Electrophysiological techniques. The current strength of a matched biphasic pulse train (50 Hz for 4 sec, 1 msec pulse width) was increased between presentations until this train evoked an intramammary pressure response similar to that induced by 0·5–1 m-u. oxytocin I.V. (Fig. 1). The current at this stage was 0·2–0·4 mA peak to peak.

Extracellular action potentials were recorded with the microtap from the supraoptic nucleus. Each spontaneously active neurone considered antidromically identified satisfied the collision test, each silent identified unit showed constant latency of the antidromic spike and frequency following two pairs of shocks at 200/sec. Some units were tested for their response to I.P. in-jections of 0·5 or 1 ml. 1·5 M-NaCl.

Microtap. The microtap has been described elsewhere (Comis, 1970; Comis, Haywood, Hodges & Hollins, 1972; Comis, Hodges & Leng, 1978). In present experiments the inner recording micro-pipette was filled with 0·15 M-NaCl and had a tip diameter of about 5 μm. The outer pipette was filled with hypertonic saline and had a tip diameter of 2–4 μm. The height of the column of solution in the outer pipette was 4–4·5 cm.

The impedance between the outer pipette and the animal was monitored with an a.c. megoh-meter. The initial resistance of an outer barrel filled with 4 M-NaCl was 1–4 MΩ: when the tap was fully closed this rose to 20–100 MΩ. The tap was opened by applying a d.c. voltage (1·25–2 kV) across piezo-electric crystals coupled to the inner barrel. The maximum voltage drew back the inner barrel by about 10 μm. This movement caused a reversible fall in the outer barrel impedance to 2–7 MΩ. The resistance of the inner recording barrel remained approximately constant at 10–15 MΩ.
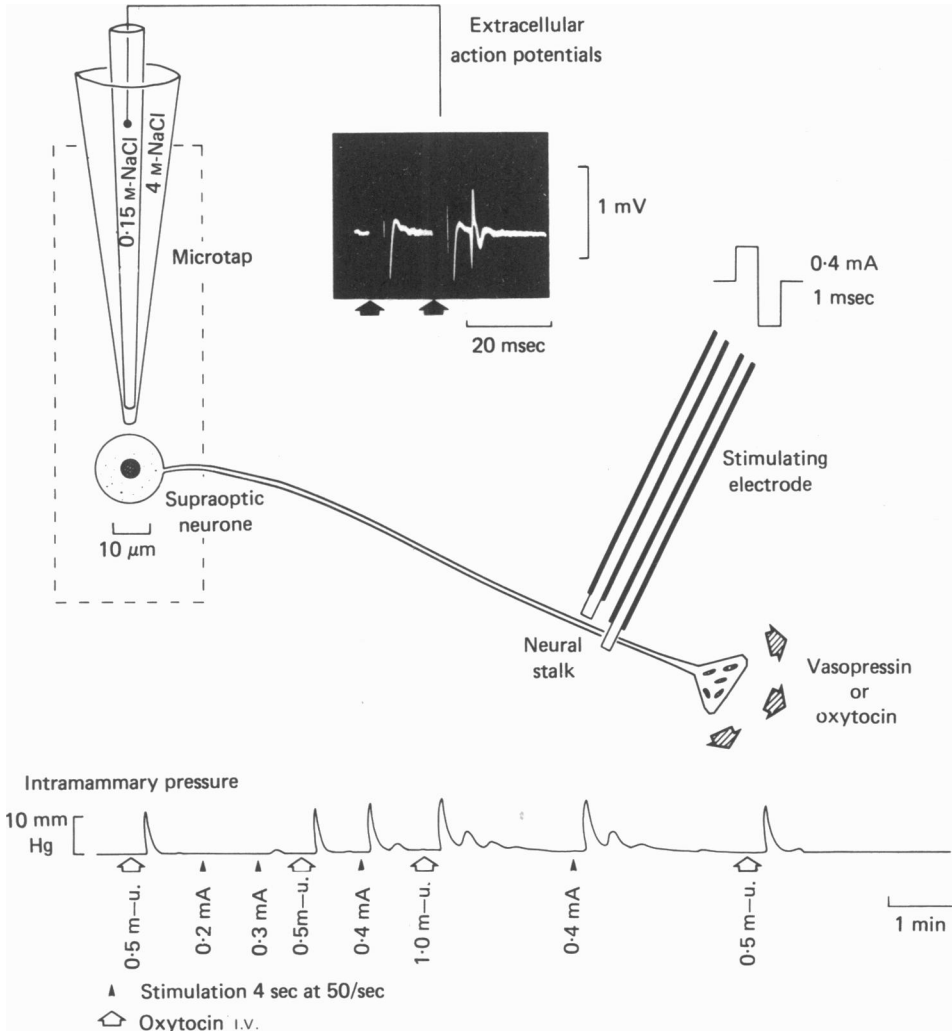
Fig. 1. Schematic diagram of the experimental arrangement. The drawing of the micro-tap illustrates the relative tip proportions of inner and outer barrels compared with the mean diameter of supraoptic neurones. The inset photograph illustrates the collision test for antidromic identification of supraoptic neurones. The photograph consists of six superimposed oscilloscope sweeps: each sweep is synchronized to a pair of stimulus pulses (arrowed) applied to the neural stalk. Each pair of pulses has been triggered by a spontaneous action potential (not shown). The antidromic spike evoked by the first of these pulses has been extinguished by collision. Similar photographs appear in Fig. 4. The record of intramammary pressure illustrates the amount of oxytocin released by trains of pulses at current intensities similar to those used for antidromic identification.
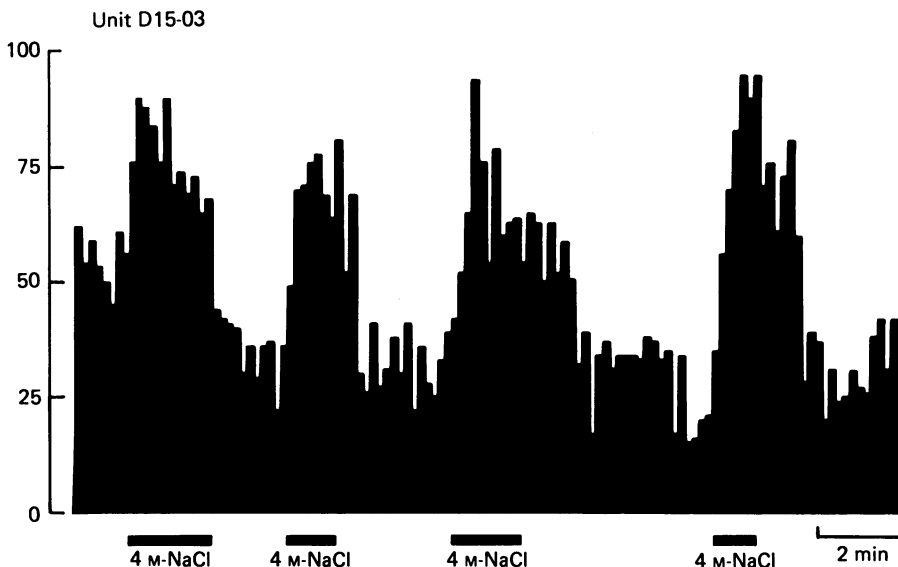
Fig. 2. The response of a continuously active supraoptic neurone to repeated microtap application of 4 M-NaCl. The mean firing rate in each 10 sec interval is plotted against time. Each application resulted in an increase in the mean level of continuous firing of the neurone. Note (1) the latency before the onset of excitation and (2) the persistence of the excitation after closing the tap, which accompany each test.

RESULTS

Of ninety-six antidromically identified supraoptic cells recorded under various conditions of plasma osmolality, thirty-seven were judged to be firing phasically, and forty-five to be continuously active (Wakerley, Poulain & Brown, 1978). Fourteen cells were firing too slowly (< 2 spikes/sec) and irregularly to be put into either of these categories. Latencies to antidromic invasion ranged from 5 to 26·5 msec, with over 90 % between 7 and 15 msec. Thresholds to antidromic activation were below 0·6 mA. The mean spontaneous firing rate, averaged over at least 4 min, of thirty-two supraoptic cells (all types) recorded before any manipulation of plasma osmotic pressure was 3·5 spikes/sec (range 0–15 spikes/sec). The plasma osmotic pressure of six rats, measured from blood samples taken at the end of experiments without I.P. saline injections, ranged between 295 and 308 m-osmole (mean 301 m-osmole).

The responses of ten supraoptic neurones, each from a different animal, were tested to I.P. injections of 0·5 or 1 ml. 1·5 M-NaCl. Each cell responded within 15 min with a sustained and substantial (> 50 %) increase in mean firing rate. These experiments confirmed that supraoptic neurones in the present preparation responded to increase in plasma osmolality in the manner described by Brimble & Dyball (1977), although the mean initial spontaneous firing rate of cells in this preparation was higher than the mean rate reported by Brimble & Dyball.

Twenty-seven supraoptic neurones were tested with up to eleven microtap applications of hypertonic saline, each application lasted between 0·5 and 4 min. Twenty-

five of these cells significantly increased their activity during each test. These changes were reversed at the end of each test. Continuously active cells responded to local hypertonic saline with a gradual acceleration in firing rate (Fig. 2). Slow, irregularly firing cells responded either in this way, or else developed 'phasic' behaviour (Fig. 3). Phasically firing cells increased their mean level of activity by a prolongation of burst length and by an increased probability of the initiation of bursts. Some phasic cells also showed an increase in intraburst firing rate.

TABLE 1. Numbers of neurones tested by microtap applications of saline, distributed by electrophysiological identification and by the strength of the test solution

	Hypertonic saline			Isotonic saline
	4 M	1·5 M	1 M	0·15 M
Supraoptic neurones				
Continuous	5	5*	3	2
Phasic	1	5	2*	2
Slow irregular	3	3	0	1
Neurones outside the supraoptic nucleus	25	10	0	0

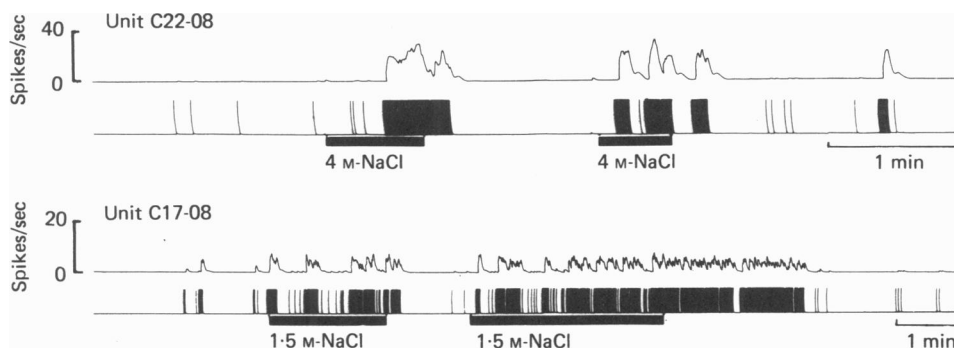* The number includes one neurone that failed to exhibit significant excitation.

Fig. 3. Polygraph records of two supraoptic neurones illustrating their response to microtap applications of hypertonic saline. These units were firing very slowly and irregularly when first encountered.

Two supraoptic cells, tested with 1 and 1·5 M-NaCl respectively, failed to show a significant increase in activity. The responsive neurones included sixteen tested at one or other of these concentrations. The remaining cells were tested with 4 M-NaCl (Table 1). Five supraoptic cells tested with microtap applications of isotonic saline were apparently unaffected (< 10 % change in firing rate).

Eight of the responsive supraoptic cells were firing phasically when first encountered. These cells were studied for 25–90 min through up to five tests of 60 sec or longer (total of twenty-five tests, mean duration 117 sec). In seven of these cells the longest burst seen in that cell began during microtap application of hypertonic saline. The microtap tests also seemed to provoke the initiation of bursts. Usually the microtap

was opened during a silent phase, and a burst often began within 10 sec. In four phasic cells such a burst ended the shortest silent interval seen in that cell (Fig. 4).

The latency to apparent excitation varied from seconds to tens of seconds. However most tests produced a response within 20 sec and nearly all within 30 sec. When pooling data the first 30 sec of each test was considered separately. The firing rate in the remainder of each test was calculated, and data from all tests on each cell were
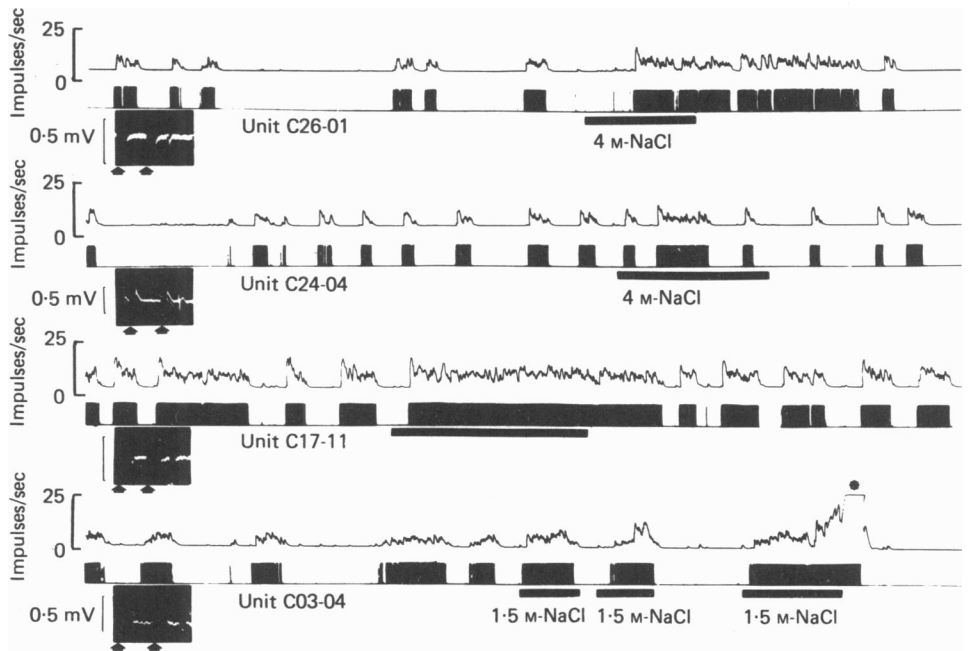

Fig. 4. Polygraph records of four supraoptic neurones illustrating their response to microtap applications of hypertonic saline. Three of these neurones were firing phasically when first encountered: unit C03.04 was firing slowly and irregularly but after repeated tests showed some 'spontaneous' bursts. The top three units show prolonged bursts of activity coinciding with microtap tests, the bottom unit shows increased intraburst firing towards the end of prolonged tests. Note the range of latencies; in the case of unit C03.04 a burst always begins within 15 sec of opening the tap, whereas in unit C26.01 the latency appears to be about 50 sec. * indicates that the firing rate exceeded 25/sec.

pooled. On the assumption that the firing rate of each cell was dictated by Poisson processes, the difference between this mean response rate and the spontaneous activity of the twenty-five responsive supraoptic cells was highly significant (difference $\gg$ 3 s.D. of the response). The mean response of sixteen of these cells was more than twice the spontaneous rate (Fig. 5).

In some cells (e.g. unit CO3.04 in Fig. 4) prolonged excitation resulted in very fast firing, and such firing occasionally appeared to result in loss of the unit. It is possible that these cells may have been totally depolarized by the local hypertonic saline.

The increased activity resulting from microtap application of hypertonic saline persisted for up to 3 or 4 min after closing the tap. However the firing rate of most cells when averaged over the periods 1–4 min after the end of each test was within 20 % of the mean rate recorded before any test. For every 'responsive' cell where at
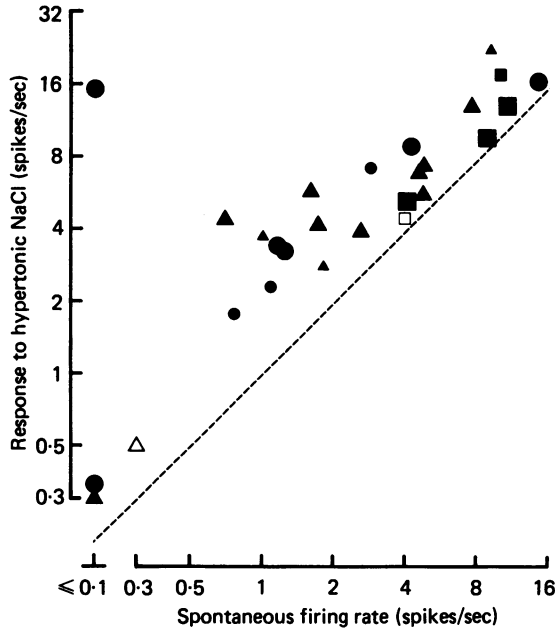
Fig. 5. The mean firing rate of each supraoptic neurone towards the end of microtap applications of hypertonic saline (i.e. excluding the first 30 sec of each test) is plotted against the initial spontaneous firing rate. Circles, squares and triangles denote neurones tested with 4, 1·5 and 1 M-NaCl respectively. Large symbols denote neurones for which data corresponding to two or more tests of over 60 sec each has been pooled: these cells also contribute to Fig. 6. Filled symbols imply that the increase in firing rate is highly significant (see text).
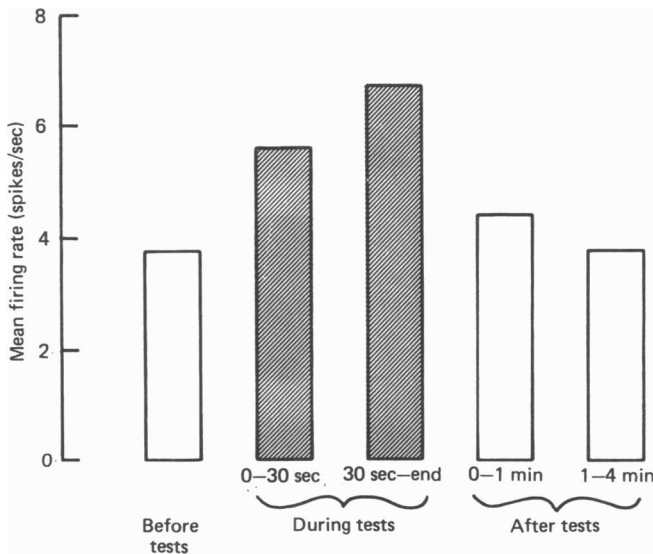

Fig. 6. Pooled data from nineteen supraoptic neurones recorded through two or more tests, each of 60 sec or more. Shaded bars are the averages of counts obtained with the microtap open, during local application of hypertonic saline. Open bars are the averages of counts obtained with the microtap closed.

least 4 min of such data is available, the firing rate was significantly lower than the firing rate during tests. Nineteen of the twenty-seven units were tested twice or more with tests of at least 60 sec. The mean profile of the response of these cells to local hypertonic saline is outlined in Fig. 6. One unresponsive cell is included in this group: this cell was tested twice before and once after an I.P. injection of 1 ml. 1·5 M-NaCl. Data from only the first two of these tests contribute to Figs. 5 and 6 since the background activity of the cell was greatly increased after the injection.

Thirty-five neurones, close to the supraoptic nucleus but not antidromically invaded, were tested with applications of hypertonic saline for 60 sec or more. One neurone tested with 1·5 M-NaCl and eight tested with 4 M-NaCl were apparently excited by one or more tests. The remainder showed less than a 20 % change in firing rate. One excited cell and five non-excited cells were tested with an I.P. injection of 1 ml. 1·5 M-NaCl, and each failed to respond to this systemic stimulus with any sustained, substantial (> 20 %) change in firing rate within 15 min of the injection.

DISCUSSION

Supraoptic neurones respond to systemic increases in plasma osmotic pressure with increased electrical activity. In particular, I.P. injections of hypertonic solutions lead to a smooth acceleration in the firing of continuously active oxytocinergic neurones, and induce in phasic, vasopressinergic cells an increase in mean level of activity that is largely the result of a prolongation of burst length (Brimble & Dyball, 1977; Brimble, Dyball & Forsling, 1978). In present experiments, both continuously and phasically active supraoptic neurones were excited by local applications of hypertonic saline. The responses observed were similar to those induced by I.P. injections of hypertonic saline. Present results are thus consistent with the hypothesis that supraoptic neurones are osmoreceptors.

It is possible that the observed effects were mediated by interneurones or by glial cells. This proviso does not affect the inference that the supraoptic nucleus contains osmoreceptors. Moreover it is not established that the rat supraoptic nucleus contains any interneurones. The rabbit supraoptic nucleus has recently been reported to contain small interneurones (Felton & Cashner, 1979). However, there has been no report of the existence in the rat supraoptic nucleus of any neurones that do not project to the neurohypophysis/median eminence.

Some cells outside the supraoptic nucleus responded to local hypertonic saline, and one of these responsive cells was unaffected by I.P. injection of hypertonic saline. Brimble & Dyball (1977) found no strong responses to I.P. hypertanic saline amongst the few cells that they tested outside the supraoptic and paraventricular nuclei. Nevertheless, some of the cells outside the supraoptic nucleus that were activated by local hypertonic saline may have been osmoreceptors, whether subserving the magnocellular neurosecretory system or some other osmoresponsive system. Alternatively the microtap stimulus may have exceeded the physiological stimulus. In this case, either the microtap stimulus exceeded any physiologically occurring changes in extracellular sodium chloride, or else not all neurones experience the changes that occur in the extracellular environment of osmoreceptors. The supraoptic nucleus is heavily vascularized (Scremen, 1970) and may experience maximal osmotic changes in consequence of its intimate connexion with the blood supply.

Raising the external osmotic pressure can stimulate transmitter release from nerve endings (Hubbard, Jones & Landau, 1968). Thus the microtap application of hypertonic saline may have released an endogenous excitatory transmitter. Acetylcholine, for example, can provoke and prolong bursts in phasic cells (Bioulac, Gaffori, Harris & Vincent, 1978). This explanation requires that local hypertonic saline did not produce similar release of inhibitory transmitter. It is possible however that the mechanism of osmoreception in the supraoptic nucleus resides in the supersensitivity of nerve endings to osmotic changes.

The fundamental question, what change in extracellular NaCl was experienced by supraoptic neurones when they responded to local hypertonic saline, cannot be answered fully. Certainly the concentration within the microtap, 1–4 M-NaCl, was unphysiological. However the amounts applied were tiny in comparison with the volume of the cell bodies. Comis, Evans & Whitfield (1964) estimated the rate of flow of solutions from the tip of an open microtap, by using a test solution of 30% radioactive labelled glutamate. With an outer barrel of 5 μm in tip diameter (which is larger than used in present experiments) flow rates were of the order of 10^{-11} to 10^{-12} mol/sec. The higher value corresponds to a total applied volume of 10^{-7} ml. over 20 sec: most supraoptic neurones responded to local hypertonic saline within this time. If in 20 sec the applied 4 M-saline diffuses no more than 25 μm from the point of application (i.e. slightly more than the diameter of a supraoptic neurone) and is confined to an extracellular space comprising 20% of total volume, then the mean increase in NaCl concentration will be of about 20% of the normal concentration. This corresponds to a supra-threshold stimulus: all supraoptic neurones responded strongly to tests with 4 M-NaCl. If the spread of hypertonic saline in 20 sec exceeds 25 μm, then the mean concentration around the neurone will be lower: if the spread is less, then the *mean* concentration around the neurone will be no greater. Thus the mean increase in osmolarity around a supraoptic cell body resulting from the microtap application of hypertonic saline is likely to be within the physiological range of osmotic changes. However, this increase will not be distributed uniformly over the cell surface, and the exposure of particular sites to unphysiological concentrations may invalidate the inferences drawn from this study. Nevertheless, the responses observed of supraoptic neurones to local hypertonic saline and the relative lack of response outside the supraoptic nucleus suggest that supraoptic neurones are osmosensitive.

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