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UNIT ACTIVITY IN RAT DIENCEPHALIC ISLANDS—THE EFFECT OF ANAESTHETICS

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SUMMARY

1. Unit activity was recorded with steel micro-electrodes from 486 hypothalamic neurones in rat diencephalic island preparations.

2. The histograms of firing frequencies for populations of hypothalamic units from unanaesthetized preparations and from those under urethane anaesthesia were not significantly different. The firing rates of both were significantly faster than those observed in intact brains under urethane.

3. The mean distance between stable units in unanaesthetized island preparations did not differ significantly from that in preparations anaesthetized with urethane.

4. The response of individual neurones to intravenous injections of urethane was variable, and apparently not associated with the onset or maintenance of anaesthesia. Some showed transient acceleration, some deceleration and some no change in rate or pattern of discharge.

5. All neurones tested were slowed or stopped by intravenous injections of subanaesthetic doses of sodium methohexitone (Brietal). The responses were highly reproducible and dose-dependent.

6. Brietal also produced a fall in arterial pressure and depressed respiration. Inhalation of amyl nitrite evoked larger hypotensive responses but did not affect unit activity; nor did inhalation of CO_2 (hypercapnia) or N_2O (hypoxia).

7. It is concluded that urethane anaesthesia is not associated with any direct action on hypothalamic neurones. The depression of firing rate in hypothalamic neurones induced by Brietal may represent an important forebrain mechanism in anaesthesia by this agent.

INTRODUCTION

In the last decade unit recording in the hypothalamus has become a favourite method of investigating the control of neurovegetative functions

(Cross & Green, 1959; Cross & Silver, 1966; Beyer & Sawyer, 1969). Although techniques of monitoring single cell activity from the hypothalamus of conscious, unrestrained animals are becoming available (Hellon & White, 1966; Beechey & Lincoln, 1969; Findlay & Hayward, 1969) most of the work so far published has been done under urethane or barbiturate anaesthesia. The results show that the hypothalamus is responsive to humoral changes in e.g. osmotic pressure, blood glucose and sex hormones, but it is important to know how the anaesthetics used in these experiments themselves influence hypothalamic neurones. Moreover, since altered activity could be due either to a direct effect of the anaesthetic or to influences transmitted from other brain regions it is desirable to isolate the hypothalamus as far as possible from afferent nervous inputs. The method of preparing diencephalic islands in rats described by Cross & Kitay (1967) meets this need. In the present experiments we have used the island preparation for a comparative study of the action on hypothalamic neurones of urethane and the short-acting barbiturate Brietal. Both agents are highly effective anaesthetics in the rat but we find they exert markedly different effects on the hypothalamus.

METHODS

Fifty-one female Wistar rats weighing 200–250 g were used. They were fed on compound pellets (Oxoid Division, Oxo Ltd.) and water *ad lib.*, and maintained at a room temperature of 21° C and artificial daylight fixed between 05.00 and 17.00 hr. Vaginal smears were taken for a period of at least 12 days and the sexual state checked at autopsy by examination of uteri and ovaries.

All the animals were anaesthetized with sodium methohexitone ('Brietal', Eli Lilly & Co. Ltd.) administered intravenously by way of a cannulated tail vein, and diencephalic islands prepared as previously described (Cross & Kitay, 1967). This involved lowering a cylindrical cutting device through the brain to enclose the hypothalamus and thalamus and removing all other cerebral structures rostral to the mid-collicular level including the portion of the hippocampus and cortex overlying the thalamus. The cavity so formed was filled with agar to support the island. The whole operation, which was performed under full visual control, lasted about 20 min, by which time the animals had recovered from the Brietal anaesthesia. The islands, vascularized from the circle of Willis, remained viable for many hours, but unit recording sessions were restricted to 6 hr.

For unit recording the animals were divided into two groups. Twenty-three were anaesthetized with urethane (1·3 g/kg) administered intraperitoneally as a 25% (w/v) solution. The remaining animals were maintained in the unanaesthetized state. Thirty-four rats were used to test the effect of intravenous injections of Brietal (0·5–3·0 mg) and/or urethane (125 mg) upon the firing rates of single cells. Only one injection of urethane could be made in each animal because of its long-lasting anaesthetic effect.

Extracellular recordings were made with steel micro-electrodes electrolytically etched to a tip diameter of 1 μ, insulated with a lacquer (E-33-N, Insl-X Company Inc., Ossining, New York) and mounted in the micromanipulator of a stereotaxic instrument (Baltimore Instruments Ltd.). The steel ear-piece of the stereotaxic

apparatus served as the indifferent electrode, and both electrodes were led into the differential inputs of an a.c. preamplifier (Grass Model P. 15) with the passband set at 0·1–10 kc. The preamplifier output was connected with the input amplifier of the top beam of a Tektronix 502 A oscilloscope and the amplified signal from the Y plates fed to loudspeaker, tape recorder, staircase integrator (similar to device used by Dyball & Koizumi, 1969) and an external trigger mechanism which was adjusted so that each action potential produced a 2 msec duration square pulse at the output. These pulses were fed into the low level preamplifier on one channel of a polygraph (Grass, Model 5C) and when required into the event socket on a Biomac 1000 signal analyser (Data Laboratories Ltd.). The polygraph was run at a sufficiently high speed to enable each action potential to produce a single well-defined excursion of the pen. This speed was dependent on the firing rate of individual cells but was often faster than 10 mm/sec. By counting up to 100 consecutive action potentials the mean firing rate of every neurone was calculated.

Other channels of the polygraph were used for monitoring time (from the internal clock of a Devices Digitimer), the integrated firing rate, arterial pressure (using a Bell and Howell Ltd. Pressure Transducer, Type 4-327-L223, connected to the right common carotid cannula) and respiration (with a Volumetric Pressure Transducer Grass Instruments Co., monitoring flow in a tracheal tube).

A Boyle's anaesthetic machine was used in eight animals to investigate the effects of hypoxia and hypercapnia on single unit activity. Hypoxia was produced by passing nitrous oxide at a flow rate of 1 l./min over the external opening of the tracheal tube and hypercapnia was elicited in a similar manner with the carbon dioxide. The effect on unit activity of a fall in arterial pressure was studied in six rats. Hypotension was produced by the inhalation of amyl nitrite vapour administered on a swab of cotton wool held close to the tracheal tube.

At the finish of each experiment a small lesion (8 μA for 20 sec) was made with the tip of the recording electrode. Cranial arterial perfusion with normal saline containing 5% potassium ferrocyanide to form a Prussian blue spot and 10% formaldehyde was made to fix the islands for subsequent histological reconstruction of recording sites (Plate 1). The perfused islands were removed from the skull and kept in formaldehyde solution. Serial frozen sections were cut at 50 μ and stained with cresyl violet.

RESULTS

A. *Unit activity in unanaesthetized island preparations*

Table 1 shows the distribution of recorded units as confirmed by microscopic examination of serial sections through the islands. Electrode penetrations were intentionally directed to the anterior and lateral areas, so while very few posterior hypothalamic neurones were included in the sample most other nuclear regions were well represented. To obtain a rough measure of the spacing of spontaneously firing units we divided the cumulative distance of all electrode tracks (367 mm) by the number of stable hypothalamic units (243) giving 0·66 units/mm. This is certainly an underestimate since units were not sought in the thalamic portions of the islands. By including in the calculation only the distance between the first and last recorded hypothalamic units in each electrode track we obtained the much higher figure of 4·3 units/mm.

From a comparatively small sample of hypothalamic neurones in rat

diencephalic islands Cross & Kitay (1967) reported an increase in the percentage of faster firing cells as compared to that obtained with the intact brain under urethane anaesthesia. This conclusion was confirmed in the much larger population of hypothalamic cells monitored in the present study. The average firing rate for all neurones was about 3/sec, and in contrast to the intact brain the percentage firing at < 1/sec did not exceed that firing at > 6/sec (Text-fig. 1).

The spontaneous fluctuations in firing rate commonly seen in association with changes from synchronized to desynchronized e.e.g. in the intact brain were conspicuously absent in the island preparations, and the neurones were uniformly unresponsive to sensory stimulation, e.g. pinching the tail.

TABLE 1. Distribution of recorded hypothalamic units

Location	Number of cells: No anaesthetic	Number of cells: Urethane
Anterior hypothalamic and medial preoptic areas	120	108
Lateral hypothalamic area	62	85
Dorsomedial and ventromedial nuclei	46	30
Paraventricular nucleus	12	13
Posterior hypothalamic area	3	7
Totals	243	243

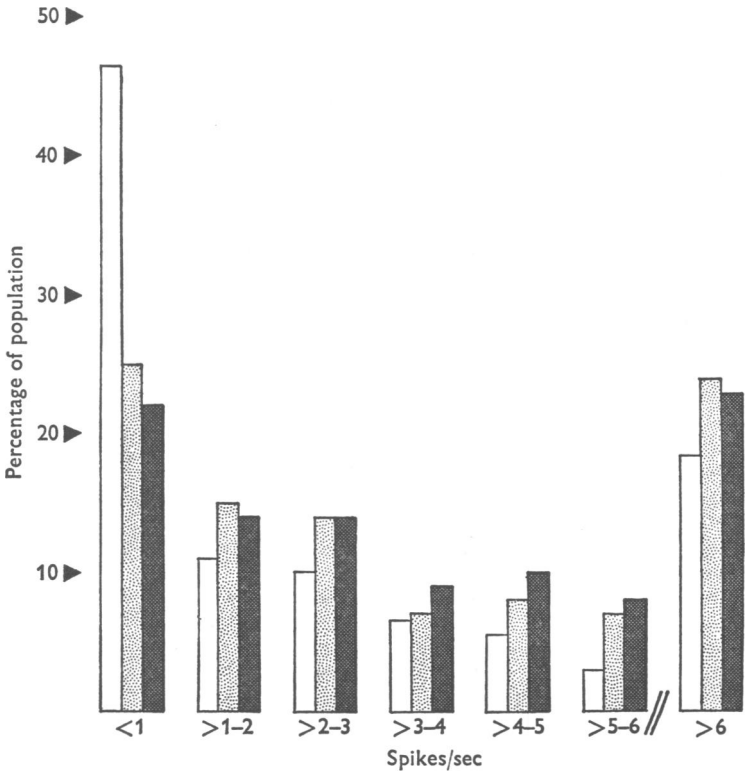
B. *Unit activity in islands under urethane anaesthesia*

As can be seen from Table 1 the location of recorded units in the island preparations under urethane was comparable to that of the unanaesthetized sample. A depressant action of urethane might be reflected in a reduction in the number of active units monitored, but this was not found. On the contrary 243 units were recorded over a total electrode track distance of 374 mm giving the result 0·65 units/mm. With the alternative method for calculating cell density, mentioned above, the figure of 3·7 units/mm was obtained. Neither of these differs significantly ($P > 1·0$) from the corresponding value in unanaesthetized rats. The extracellular wave forms of units recorded under urethane showed no obvious differences from those obtained from unanaesthetized preparations (Text-fig. 2), and the mean signal-to-noise ratio was about 8:1 in both groups.

Text-fig. 1 shows histograms of the unit firing frequency in unanaesthetized and anaesthetized preparations from which it is apparent that the two populations of hypothalamic units have markedly similar distributions. The Kolmogorov–Smirnoff test applied to these histograms confirmed that they do not differ significantly ($P > 0·5$).

To see if the similarity of firing frequency might be masking alterations

of spike patterning we used the technique of Tokizane & Eldred (unpublished, cited by Bullock & Horridge, 1965). For each cell this compares the mean interspike interval with the standard deviation of the intervals expressed as a percentage of the mean. Forty-eight cells were used in this analysis, equally distributed between the unanaesthetized and urethanized animals. One hundred consecutive interspike intervals were measured in each case and the two groups compared. No significant difference was found ($P > 1{\cdot}0$).



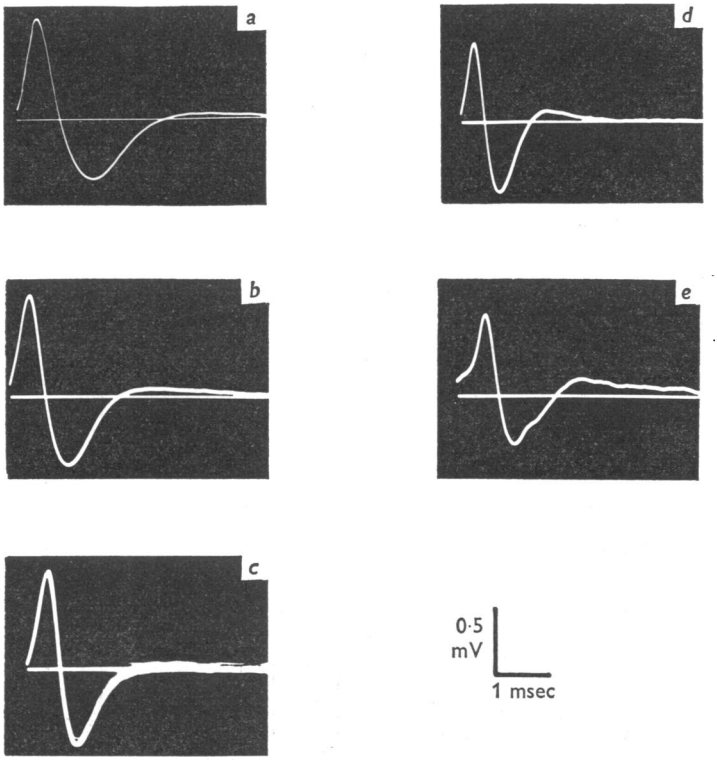
Text-fig. 1. Histograms of firing frequency distributions of neurone populations from islands in rats with (■, 243 cells) and without (▦, 243 cells) urethane anaesthesia, contrasted with the slower firing population of hypothalamic neurones from intact brains (□, 618 cells) of rats under urethane.

C. *Effect of intravenous urethane and Brietal on single units*

The advantage of this approach was that each cell acted as its own control, being monitored before, during and after the induction of anaesthesia. The injection of 125 mg urethane as a 25% (w/v) solution caused loss of limb withdrawal reflexes within 1½ min, the abolition of all spontaneous movement and sometimes a slight fall in arterial blood pressure

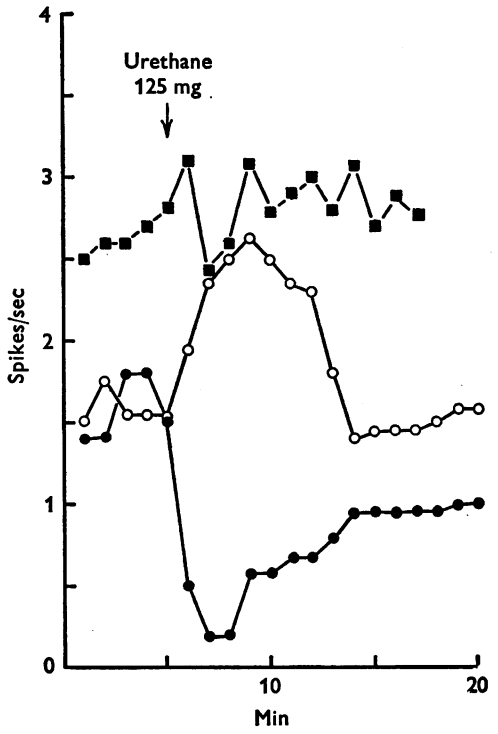
(< 10%). The anaesthetic action of this dose level persisted for many hours.

Individual hypothalamic units responded to intravenous injections of urethane in a variable manner (Text-fig. 3) and, due to the long-lasting anaesthetic effect, only one test was possible in each rat. Altogether twenty-one experiments were performed. In ten cases the injection was followed by a reduction in firing rate of greater than 10%. In four of these

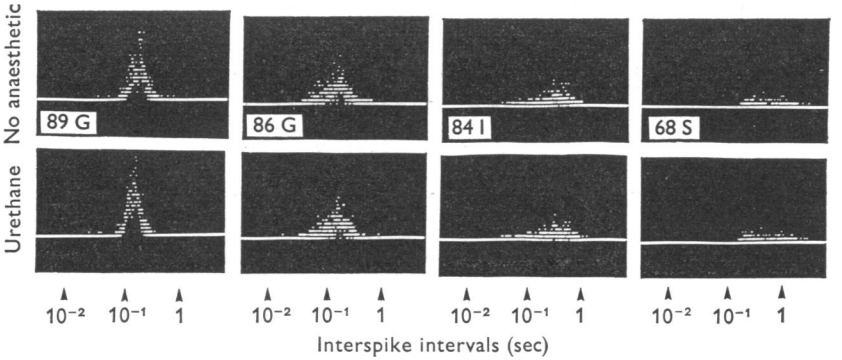


Text-fig. 2. Typical wave forms of action potentials of hypothalamic neurones recorded from diencephalic islands. *a* and *b* recorded under urethane, *c*, *d* and *e* without anaesthesia. *c* comprises six superimposed sweeps.

animals the rate began to return to normal after several minutes. In a further six tests the opposite results occurred, viz. an acceleration of unit firing. In the remaining five experiments no obvious change (< 10%) occurred. With this last group the distribution of interspike intervals was studied with the Biomac 1000. As can be seen in Text-fig. 4 the interval distributions for 2 min periods before injecting the urethane and after the onset of anaesthesia are remarkably similar for each cell. It seems unlikely



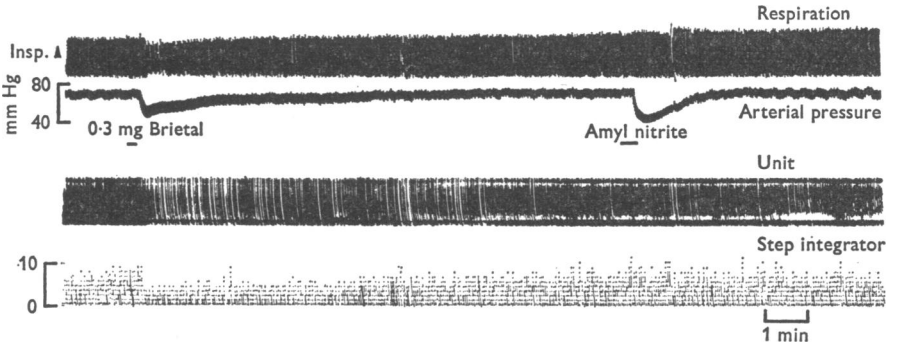
Text-fig. 3. Graph showing the variable response of three hypothalamic neurones from different island preparations to intravenous injection of an anaesthetic dose of urethane.



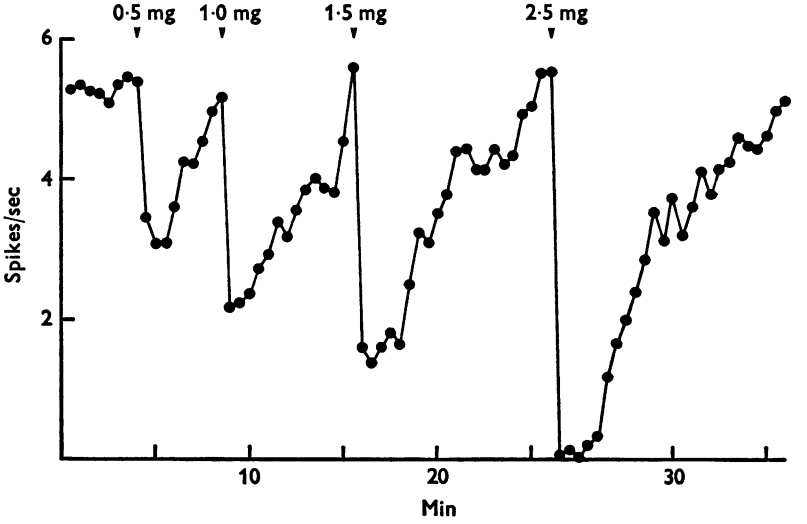
Text-fig. 4. Photographs of oscilloscope displays of Biomac 1000 computations of interspike interval distributions from four hypothalamic units over 2 min periods before intravenous injection of urethane and after the onset of anaesthesia. Note the clear difference between cells and the lack of change after urethane.

therefore that the urethane was inducing a coded change in the impulse trains.

In contrast to the uncertain response to intravenous urethane, injections of sodium methohexitone produced highly consistent effects. Forty-five



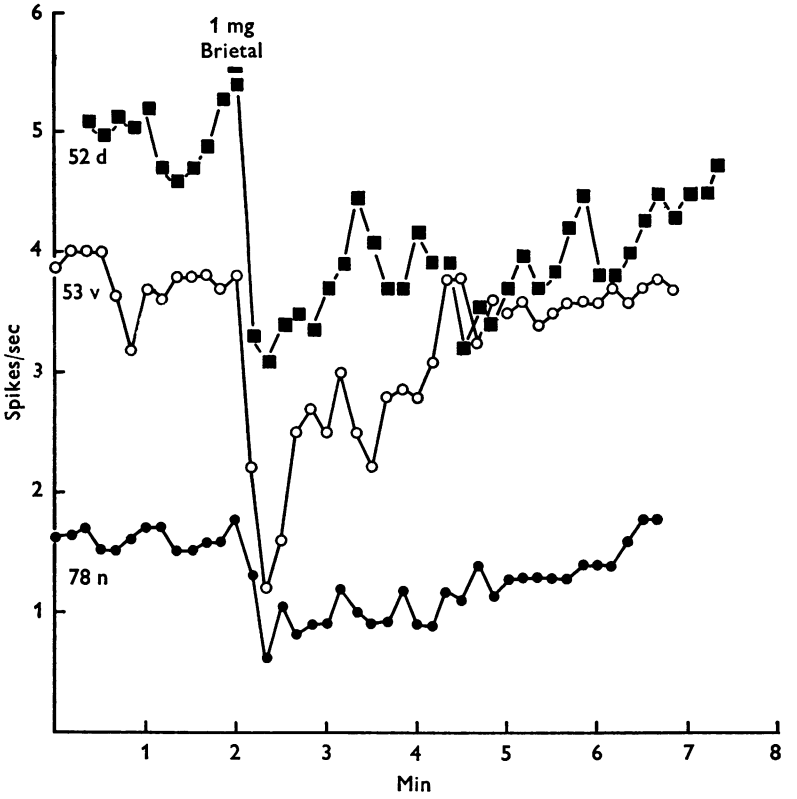
Text-fig. 5. Polygraph record comparing the effects of Brietal and amyl nitrite on respiration, arterial pressure and the firing rate of a hypothalamic neurone. Despite a larger hypotensive effect amyl nitrite did not change the firing rate though Brietal produced a definite slowing of the unit.



Text-fig. 6. Graph showing graded responses of a hypothalamic neurone to intravenous injections of sub-anaesthetic doses of Brietal.

neurones were tested with injections of 0·5–3·0 mg Brietal in thirty-four rats. In every case, after a latency of 5–10 sec, the spontaneous discharge of the cell was slowed or arrested. At the same time there was invariably a fall in arterial pressure, and frequently a reduction in the rate and/or depth of respiratory movements (Text-fig. 5). The reduction in cell firing

rate persisted for 0·5–10 min and the magnitude of this effect was dose dependent (Text-fig. 6), although individual neurones varied in their apparent sensitivity (Text-fig. 7). The amplitude and wave form of the recorded action potential remained constant during the period of depression and was unaltered even during recovery from a period of complete inhibition. This fact gave assurance that the same cell was being recorded throughout the experiment.

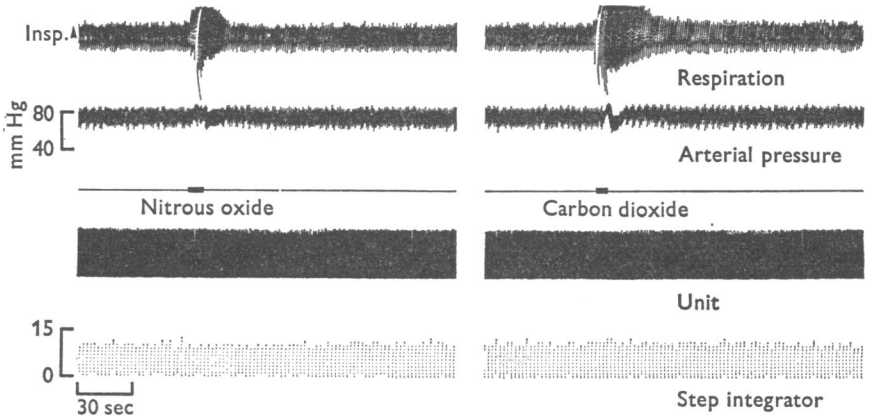


Text-fig. 7. Graphs showing the depressant effects of intravenous injection of 1 mg Brietal on the firing rate of three hypothalamic neurones.

Control injections of normal saline were given in thirty-two experiments and were without effect. Inhalation of the vasodilator, amyl nitrite, produced a fall in arterial pressure ranging from 20 to 50 % and by controlling the duration of exposure to the vapour a hypotensive response was produced similar to or greater than that following intravenous barbiturate injections. In twelve cells tested in this way there was no reduction in firing rate when the mean fall in arterial pressure was 40 %, whereas they

were all greatly slowed by intravenous doses of 0·5–1·0 mg Brietal when the mean fall in arterial pressure was only 25% (Text-figs. 5, 9).

The reduced respiratory rate and fall in tidal volume after intravenous barbiturate (Text-fig. 5) might also have contributed to the depression of unit discharge through a resultant hypoxia and/or hypercapnia. This possibility was examined by testing the response of cells to inhalation of nitrous oxide or carbon dioxide. In thirty-five tests on fifteen cells hypercapnia failed to alter firing rates although in every case a marked hyperpnoea was induced. Similarly thirteen cells were unaffected in twenty-one tests with hypoxia (Text-fig. 8). Even the combination of hypercapnia and hypotension did not change firing rate (Text-fig. 9).



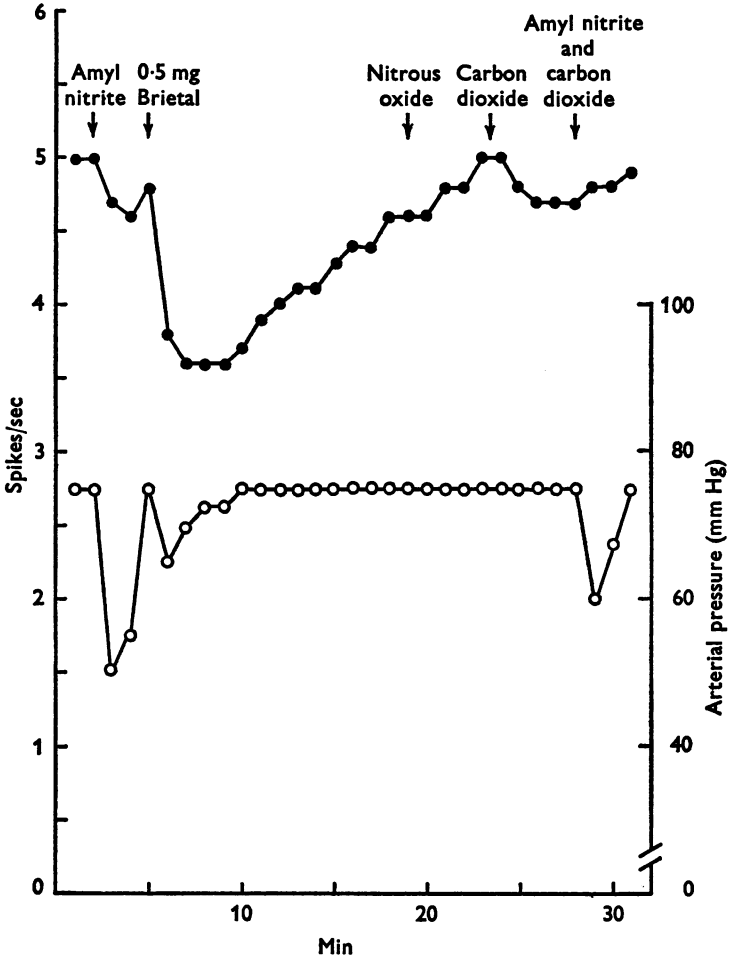
Text-fig. 8. Polygraph record showing the negligible effect of inhalation of nitrous oxide and carbon dioxide on the firing rate of a hypothalamic neurone. Note the hyperpnoea induced by hypoxia and hypercapnia.

DISCUSSION

Urethane, ethyl carbamate, is widely used as an anaesthetic in research laboratories but given alone it is only suitable for acute experiments since it causes a number of undesirable sequelae. These include pulmonary oedema, agranulocytosis and liver intoxication. In addition to prolonged anaesthesia the acute effects are also considerable. There is enhanced adrenaline output (Aub, Bright & Forman, 1922), hyperglycaemia (Conybeare, Densham, Maizles & Pembrey, 1927) and prolonged hypersecretion of ACTH (Spriggs & Stockham, 1964). Its mode of anaesthetic action is obscure but Crescitelli (1948) showed that urethane causes hyperpolarization in the isolated sciatic nerve of the frog and Crawford & Curtis (1966) found that in the unanaesthetized cat *cerveau isolé* preparation micro-iontophoretic injection of large doses of urethane depressed the firing of some cells in the precruciate cortex. Recent work by Angel &

Unwin (1970) has revealed that this anaesthetic impairs transmission through the ventrobasal thalamus.

Our data show that there is no direct effect upon the hypothalamus as judged by the unchanged values for the number of stable units recorded



Text-fig. 9. Graphs of arterial pressure and firing rate of a hypothalamic neurone comparing the effects of Brietal with those of hypotensive, hypoxic and hypercapnic stimuli, alone and in combination.

per millimetre of electrode track, the firing frequency histograms and the interspike interval analyses. We think that more importance should be attached to the data collected from cell populations (Text-fig. 1) than to the variable effects of intravenous injections of urethane (Text-fig. 3). This is because the intravenous studies reflect activity for no more than the first 20 min of an anaesthesia maintained for many hours. On the other

hand the cells used in the population histograms were obtained for up to 6 hr after the injection of urethane and are more representative of neural activity during anaesthesia. It is still possible that urethane modulates the activity of hypothalamic neurones in the intact brain through afferent inputs from other regions. The increased discharge rate of island cell populations with or without urethane, as compared to that obtained from the intact animal, indicates the probability that afferent impulses do modify the intrinsic activity. Clearly either brain stem or spinal structures must be affected since limb withdrawal reflexes are abolished by urethane in the decerebrate preparation. At all events it would seem that earlier single unit studies on the rat hypothalamus (Barraclough & Cross, 1963; Cross & Silver, 1965; Lincoln & Cross, 1967) may not have been greatly prejudiced by the use of urethane anaesthesia.

Sodium methohexitone is a short-acting barbiturate providing surgical anaesthesia for about 5 min whereupon a further dose is usually required. This short duration is due to dilution in the blood after intravenous injection (Welles, McMahon & Doran, 1963) and to rapid detoxication by the liver. Recovery is complete and rapid (under 30 min) with no reported side effects. It is extensively used in clinical medicine (Coleman & Green, 1960).

Unit studies have been carried out under barbiturate anaesthesia (Brooks, Ushiyama & Lange, 1962; Anand, Chhina, Sharma, Dua & Singh, 1964; Dafny, Bental & Feldman, 1965) and these may suffer from a loss of detectable cells or a reduction in firing activity, as has been reported by previous workers (Stuart, Porter, Adey & Kamikawa, 1964; Terasawa & Sawyer, 1969). In our experiments intravenous injections of Brietal have produced clear and unambiguous results (Text-figs. 6 and 7). Without exception the firing rate of single units was depressed, and as little as 10% of the anaesthetic dose was sufficient to give reproducible effects. With anaesthetic doses the duration of the action on cell firing corresponded roughly to the duration of the anaesthetic effect. It seems likely therefore that this general depression of neuronal firing in the hypothalamus represents an important forebrain component of Brietal-induced anaesthesia in the intact animal. On the other hand the hypothalamus certainly cannot be the only site of action of the barbiturate for in the decerebrate island preparations more caudal central nervous structures must be responsible for the accompanying loss of spinal reflexes and respiratory changes.

In the light of the immediate and precipitous effect of intravenous Brietal on the activity of hypothalamic neurones the lack of response to substantial reductions in arterial pressure, to hypoxia and to hypercapnia (Text-fig. 9) calls for comment. Komisaruk, McDonald, Whitmoyer &

Sawyer (1967), recording from the thalamus in rats under urethane anaesthesia, found that hypotension induced by amyl nitrite elicited an e.e.g. arousal and a considerable increase in multi-unit activity. We observed no responses in the neurally isolated hypothalamus though it is very unlikely that a mean fall in arterial pressure of 40% could occur without diminished blood flow through the island. It would appear that the discharge rates of deafferented hypothalamic neurones are to a large extent independent of fluctuations in blood flow. They are also surprisingly resistant to changes in oxygen or carbon dioxide tension (Text-figs. 8, 9). Cross & Silver (1963) working with intact rabbits under urethane anaesthesia reported that the majority of single units in the thalamus and hypothalamus were excited non-specifically by strong hypercapnic stimuli and a lower proportion by hypoxia. We cannot of course extrapolate directly from the earlier rabbit experiments to those reported here in rat island preparations. Nevertheless from the magnitude of the respiratory change observed (Text-fig. 8) we think that plasma P_{O_2} and P_{CO_2} must have undergone comparable changes. Indeed, in a few recent tests with intact rats under urethane we obtained hypothalamic unit responses to hypoxia and hypercapnia quite similar to those previously observed in the rabbit. We now think, contrary to earlier views (Cross, 1964; Cross & Silver, 1963) that direct chemosensitivity of hypothalamic neurones to P_{O_2} and P_{CO_2} may be minimal and that responses to changes in these humoral factors are probably due to incoming afferent impulses from the brain stem.

The results we have described in this paper underline the usefulness of the diencephalic island preparation for studying direct humoral effects on neurones of the hypothalamus. It has certain advantages over the microiontophoretic injection technique. Any substance that is soluble in blood plasma can be tested, and reaches the neurones by the physiological route, without leap-frogging the blood–brain barrier. The technique also avoids the complications of anaesthesia and afferent nervous input from other brain regions. We have found it useful in gaining information about the interaction of anaesthetics and endogenous hormonal state. For example, a well-defined periodicity in the firing rate of hypothalamic neurones during the rat oestrous cycle was unaffected by the presence or absence of urethane anaesthesia (Cross & Dyer, 1970*a*). On the other hand a striking depression of unit activity in hypophysectomized female rats (Cross & Dyer, 1970*b*) was associated with a marked increase in susceptibility to the anaesthetic action of intravenous Brietal. Having now examined the effects of the anaesthetics most commonly used in unit recording from the hypothalamus, we intend to devote future work with the island technique to the further analysis of neuroendocrine mechanisms.

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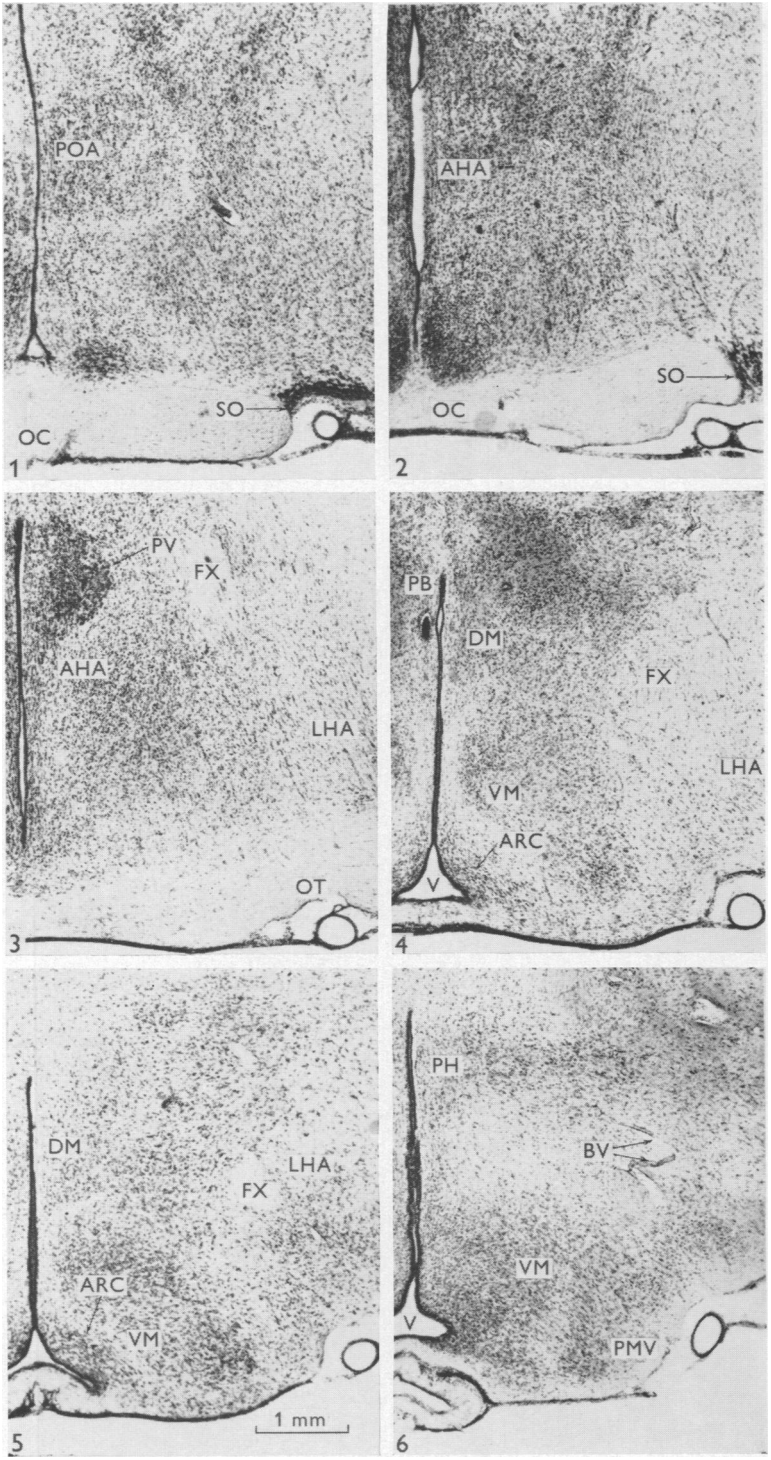
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EXPLANATION OF PLATE

PLATE 1

Frontal sections at 0·5 mm intervals through hypothalamic island fixed by carotid perfusion after 6 hr of unit recording. 50 μ frozen sections stained with cresyl violet. AHA, anterior hypothalamic area; ARC, arcuate nucleus; BV, blood vessel; DM, dorsomedial nucleus; FX, fornix; LHA, lateral hypothalamic area; OC, optic chiasma; OT, optic tract; PB, Prussian blue spot; PH, posterior hypothalamic area; PMV, ventral premammillary nucleus; POA, preoptic area; PV, paraventricular nucleus; SC, suprachiasmatic nucleus; SO, supraoptic nucleus; V, third ventricle; VM, ventromedial nucleus.