such a time that the animals awakened at approximately the same time as those given pentobarbitone by infusion (16.7 ± 3.2 (4); $P < 0.01$). Because of the high mortality (>60%) occurring during the infusion and the possible presence of barbiturate metabolites in the brain extract this experiment was not extended to include other drugs.

Secondly, female rats were kept anaesthetized for approximately 12 h by repeated i.p. injections of pentobarbitone. A typical dose regime was 25 mg/kg followed 2, 5 and 10 h later by further injections of 20 mg/kg. The next day, approximately 12 h after animals had regained their righting reflex, the duration of anaesthesia and brain barbiturate level on awakening was determined following an i.c.v. injection of 800 μg [^{14}C]-pentobarbitone. Compared with saline pre-treated control animals, pentobarbitone-treated animals slept for a shorter period of time (control 6.9 ± 1.8 (7); treated 4.0 ± 1.7 (6) min. $P < 0.01$) and awakened with higher brain barbiturate levels (control 21.7 ± 7.7 (7); treated 35.2 ± 13.2 (6) μg/g. $P < 0.05$).

However, without using large numbers of animals, it is not possible to determine the time course of such change in CNS sensitivity. We therefore decided to measure the duration of halothane-induced anaesthesia in drug pre-treated animals in the hope that the change in CNS excitability might be more easily followed. Repeated injection of pentobarbitone for 10 h, as outlined above, resulted in the development of tolerance to halothane which was maximal 24 h after the last pentobarbitone injection. Tolerance was followed by a 'rebound' hypersensitivity to halothane which reached a maximum 42 h after pentobarbitone. Sensitivity had returned to normal by the third day. Similar patterns of change in sensitivity to halothane, i.e. a post-tolerance hypersensitivity, were also observed after repeated injections of amylobarbitone and meprobamate. It is hoped to extend this work to include other centrally active drugs, particularly those on which physical dependence may develop.

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Effects of dexamethasone on phenylethanolamine N-methyltransferase (PNMT) and adrenaline (A) in the brains of adult and neonatal rats

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Recently the regional distribution of PNMT in rat brain has been demonstrated by immunohistofluorescence (Hökfelt, Fuxe, Goldstein & Johansson, 1974) and biochemical techniques (Saavedra, Palkovits, Brownstein & Axelrod, 1974). Only small amounts of A have been detected in rat brain stem (Gunne, 1962). The purpose of this study was to determine (1) the distribution of PNMT in rat brain; (2) the amounts of A in those regions rich in PNMT; (3) the sensitivity of PNMT and A to glucocorticoid hormone treatment.

Sprague-Dawley rats, both newborn and adult, were used. PNMT was assayed by a modification of the method of Axelrod (1962). For catecholamines, an enzymatic method capable of detecting picogram quantities of amine was employed (Cuello, Hiley & Iversen, 1973). The sensitivity for A was 50-75 pg, amounts that gave values of twice the blank. Dexamethasone was injected s.c. (1 mg/kg for adults and 0.1 mg/kg for newborn rats).

Highest PNMT activity was recorded in the medulla in a region bounded by a transverse section at the obex and a transverse section 3 mm rostral to the obex (0.454 ± 0.052 p mol product.mg wet wt.$^{-1}$ h^{-1}, mean ± s.e.m. n = 4). The hypothalamus contained 0.194 ± 0.012 p mol product.mg wet wt.$^{-1}$ h^{-1}. Small amounts of activity were recorded in midbrain, pons and spinal cord, but none in olfactory bulb, olfactory tubercle, septum, striatum, cerebellum, cerebral cortex or hippocampus. Only traces of A could be detected in the medulla, whereas the hypothalamus contained 0.04 ± 0.01 μg/g wet wt. (mean ± s.e.m. n = 4).

Dexamethasone treatment of adult male rats daily for 13 days increased PNMT both in the

medulla (from 0.440 ± 0.092 to 0.792 ± 0.100, $P < 0.05$, $n = 6$) and in the hypothalamus (from 0.168 ± 0.056 to 0.372 ± 0.072, $P < 0.05$, $n = 6$). In the hypothalamus, A increased from 0.05 ± 0.01 to 0.08 ± 0.01 μg/g wet wt. ($P < 0.05$, $n = 6$). No change was seen in noradrenaline or dopamine concentrations of medulla or hypothalamus. A single dexamethasone injection did not change PNMT after 24 hours. Five daily injections increased PNMT by 56%, but only in the medulla. Five daily injections of dexamethasone to newborn rats had no effect on PNMT in hypothalamus or medulla, but caused a 14-fold increase in PNMT and a 30-fold increase in A in the superior cervical ganglion.

These observations are consistent with the hypothesis that the cell bodies of PNMT and adrenaline containing neurones are present in the medulla and that they send axons to terminate in the hypothalamus. The PNMT contained in the cell bodies appears to be inducible by glucocorticoid hormone, and the induced enzyme is transported to the hypothalamus, resulting in an increased A content.

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Release of ^{3}H-(−)-noradrenaline from guinea-pig hypothalamic slices: effects of adrenoceptor agonists and antagonists

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The release of noradrenaline from peripheral adrenergic nerves in response to electrical stimulation is enhanced by α-adrenoceptor antagonists and reduced by α-adrenoceptor agonists. These effects have been attributed to the presence of α-adrenoceptors on the terminal axons through which transmitter release is inhibited. The present experiments were designed to investigate whether the release of noradrenaline from hypothalamic slices of the guinea-pig was similarly affected by drugs acting on α-adrenoceptors.

Slices of hypothalamus were incubated with ^{3}H-(−)-noradrenaline, then repeatedly washed with Krebs-Henseleit solution until the rate of efflux of tritium from the tissue had fallen to a low level (approx. 1 h): then, the residual tritium was present as ^{3}H-noradrenaline. Stimulation of the slice with biphasic pulses at 10 Hz for 30 s resulted in an increase in tritium efflux. With successive periods of stimulation at 30 min intervals, there was a steady decrease in stimulation-induced efflux.

The effects of drugs on resting and stimulation-induced effluxes of tritium were determined by adding them 19 min before the second period of stimulation. When α-adrenoceptor agonists were used, cocaine (100 μM) was present to prevent their uptake and the displacement of labelled noradrenaline.

Noradrenaline decreased stimulation-induced efflux in a concentration-dependent manner in the range 2-50 μM. Dopamine was approximately equipotent with noradrenaline; adrenaline was less potent, the threshold concentration being above 5 μM; isoprenaline had no significant effect in concentrations up to 50 μM.

Piperoxane (10 μM) increased stimulation-induced efflux, but phenoxybenzamine (10 μM) and phentolamine (10 μM) had no effect. However, phentolamine (5 μM) antagonized the effect of noradrenaline (20 μM) in reducing stimulation-induced efflux.

The findings suggest the presence in the hypothalamus of α-adrenoceptors through which stimulation-induced release of noradrenaline from stores in the tissue can be inhibited, but feed-back modulation of release by transmitter noradrenaline impinging on these receptors appears to be less important than in peripheral adrenergic neurones.

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