out on each area independently. Subcellular fractions were prepared from a 10,000 *g* pellet on a discontinuous sucrose gradient based on a method by Marchbanks & Whittaker (1967) and purity of fractions ascertained by electron microscopy. Radioactivity of each sample was measured by liquid scintillation counting. Subsequent experiments measuring the ability of isolated subfractions to accumulate CPZ were attempted by incubating the subfractions in medium containing 10^{-4} M ^{3}H-CPZ at 37°C for 15 minutes. The reaction was terminated by centrifugation, the supernatant decanted, tubes wiped dry and pellet resuspended in 10% Triton X100. Samples were counted by liquid scintillation counting as in the earlier experiments.

A time course study following the injection of CPZ indicated an initial rise in all areas of rat brain rising to a maximum at about 15 min and then levelling off from 20-30 min, the highest concentration accumulating in the cortex and the smallest in the hind brain. There were surprisingly high levels in the membrane/myelin fraction compared to that in the synaptosomes in all areas, which was apparent after 15 minutes. It was found that there was a differential distribution of CPZ in the subfractions in the different areas at 10 min and 60 min after injection. At 10 min a relatively high concentration was found in the mitochondria which may be explained by our earlier work (Livingston & Phillips, 1974) on the effects of CPZ on oxidative phosphorylation in synaptosomes and mitochondria, although after 60 min the highest concentrations were found in the membrane/myelin fraction.

Experiments in which CPZ was incubated with subcellular fractions for 32 min showed similar results to whole animal experiments incubated for 60 min, the main difference being in the hind brain where there was a high accumulation in the synaptosomes relative to the other subfractions. However, in the cortex and mid-brain the previous finding of a high concentration in the membrane/myelin subfraction is reinforced, there being approximately twice as much CPZ accumulated in this fraction as in the synaptosomes and mitochondria in the cortex.

The relatively high concentration of CPZ found in the membrane/myelin fraction poses an interesting question as to the site of action of this drug in the central nervous system.

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Change in sensitivity to pentobarbitone and halothane induced by acute administration of central nervous system depressant drugs

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A withdrawal hyperexcitability, as manifested by a reduction in the duration of anaesthesia produced by an intracerebroventricular (i.c.v.) injection of pentobarbitone is seen in rats abruptly withdrawn from a number of central nervous system (CNS) depressant drugs following their chronic administration (Stevenson & Turnbull, 1974). However, since such experiments are necessarily time-consuming and because each animal can only be used once, we have investigated the possibility that CNS excitability might be altered by administration of large doses of depressant drugs over a much shorter period of time and have assessed the usefulness of other indices of CNS excitability.

First we attempted to keep male rats anaesthetized for approximately 8 h by beginning an i.p. infusion of pentobarbitone (30 mg kg^{-1} h^{-1}) immediately animals had lost their righting reflex following an i.p. injection of 50 mg/kg pentobarbitone. The rats were killed on awakening and the brain barbiturate levels were determined. Higher brain levels (23.6 ± 1.7 (5) mean ± s.d. μg/g) were found in these animals compared with rats killed on awakening after a single i.p. injection of 50 mg/kg administered at

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