DMPP. During full tachyphylaxis to 5-HT normal responses to noradrenaline, DMPP and tyramine could be obtained. In contrast, with the hearts desensitized to DMPP, responses to 5-HT were also abolished, although those to noradrenaline and tyramine were little affected. Colchicine (10^{-4} to 10^{-3} g/ml) inhibited responses to 5-HT and DMPP concentration-dependently, but had no significant effects on responses to noradrenaline or tyramine.

After the cardiac noradrenaline stores were labelled by perfusion with ^{3}H-(−) noradrenaline (10 ng/ml–Starke, 1971), bolus injections of 5-HT (512 μg), tyramine (40 μg) and DMPP (40 μg) evoked tritium release from the hearts. The pattern of tritium appearance in the perfusate after 5-HT showed a peak, 10-20 s after the injection with little release being evident after 1 minute. A qualitatively identical pattern was obtained with DMPP. In contrast, tyramine released tritium at a constant rate during the 3 min period immediately following the injection. Reducing the Tyrode calcium ion concentration from 3.6-0.2 mEq/l did not affect the tritium release after tyramine, although the release evoked by 5-HT and DMPP was markedly inhibited.

The results confirm the suggestion that 5-HT stimulant responses on the rabbit heart are the result of noradrenaline release. They further suggest that the site of release is the terminal sympathetic nerve network. The mechanism of release shows more similarities to the DMPP release mechanism (depolarization) than to that of tyramine (neuronal uptake and stoichiometric displacement).

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5-Hydroxytryptamine synthesis in the isolated perfused rat brain

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Isolated perfused organs provide an experimental system for the study of metabolism in an organ retaining its structural integrity. Little work has been done upon monoamine metabolism in the perfused brain. We have developed a method for the *in situ* perfusion of rat brain which excludes the influence of extracranial tissues on tryptophan metabolism and this paper describes the use of this method in the study of 5-hydroxytryptamine metabolism.

Rat brains were perfused using the method described by Woods, Graham & Grahame-Smith (1974). The metabolic, and histological properties of this preparation are very similar to those observed *in vivo*. For example, the rate of glucose uptake was 0.79 μmol min^{-1} gram^{-1} in the presence of 5 mmol/l glucose, and the rate of acetoacetate uptake was concentration dependent being 0.14 μmol min^{-1} gram^{-1} after loading with 1 mmol/l acetoacetate and 0.24 μmol/min^{-1} gram^{-1} with 2 mmol/l.

For the study of 5-HT synthesis rats were anaesthetized with Nembutal (60 mg/kg i.p.) and the brains perfused with a medium containing glucose (10 mmol/l) together with tranylcypromine (1 mmol/l) and tryptophan (0.1 or 1.0 mmol/l). After perfusion for varying times the brains were rapidly removed from the skull and stored at −20° C before determination of 5-HT and tryptophan concentrations.

Anaesthesia and preparation of the brain for perfusion resulted in a small increase in brain 5-HT concentration when compared with brains obtained after cervical dislocation (from 0.46 to 0.53 μg/g).

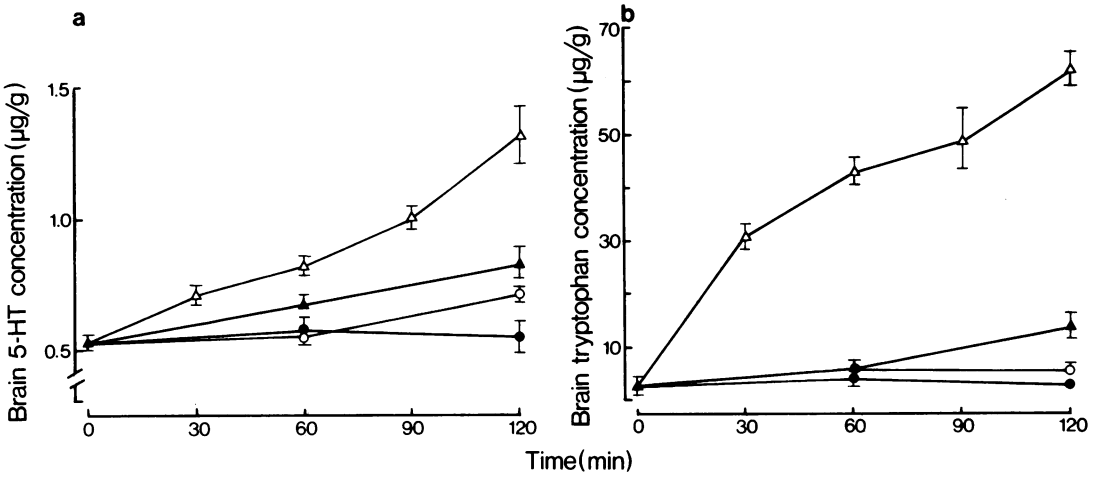

Figure 1 Brain 5-Hydroxytryptamine (Figure 1a) and L-tryptophan (Figure 1b) concentrations in the isolated perfused rat brain. Brains were perfused with either the basal medium which contained glucose (10 mmol/l) alone (●—●) or a medium containing tranylcypromine (1 mmol/l) (○—○). In other experiments the medium contained tranylcypromine (1 mmol/l) + L-tryptophan (0.1 mmol/l) (▲—▲) or tranylcypromine (1 mmol/l) + L-tryptophan (1.0 mmol/l) (△—△). Each point represents the mean ± S.E.M. for 3-7 observations.

Brain 5-HT concentrations were maintained at the control values (0.53 μg/g) during perfusion for 2 h with the basal medium which contained no added tranylcypromine or tryptophan (Figure 1a) (the initial tryptophan concentration being < 2 μg/ml). Addition of tranylcypromine (1 mmol/l) caused increase in brain 5-HT during the second h at a rate of 0.16 $\mu g\, g^{-1}\, h^{-1}$. When tryptophan was added with tranylcypromine the rate of 5-HT accumulation increased with tryptophan concentration being 0.16 $\mu g\, g^{-1}\, h^{-1}$ with 0.1 mmol/l and 0.4 $\mu g\, g^{-1}\, h^{-1}$ with 1.0 mmol/l.

Following perfusion with tryptophan the brain tryptophan concentration rose, the rate of accumulation increasing with the increasing initial concentrations (Figure 1b).

These results compare favourably with rates of 5-HT accumulation measured *in vivo* following loading with 100 mg/kg tryptophan. This model provides a means of studying brain 5-HT metabolism in a situation where there is a high degree of control over the experimental conditions.

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Distribution of chlorpromazine and its metabolites in subfractions of rat brain

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The distribution of chlorpromazine (CPZ) in the central nervous system was measured autoradiographically in the cat by Cassano, Sjöstrand & Hansson (1965) using large amounts of ^{35}S-CPZ. Earlier work by Sjöstrand, Cassano & Hansson (1965) investigating whole body distribution of CPZ in mice showed a concentration of label in the cerebral and cerebellar cortex. In order to study the area and subcellular distribution, rats were injected (8 mg/kg i.p.) with ^{3}H-CPZ at various times before being killed. Brains were removed, divided into cortex, mid-brain and hind-brain, all subsequent procedures being carried