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The effect of normetanephrine on brain levels of noradrenaline in mice

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IT HAS recently been shown that, on injection into the cerebral ventricles of mice, normetanephrine produces behavioural changes qualitatively like those produced by the parent compound noradrenaline.[1] Assessed in terms of decreased spontaneous motor activity, normetanephrine possessed approximately one quarter of the potency of noradrenaline, while in producing motor incoordination, normetanephrine was only one-tenth as potent as noradrenaline.

The preliminary experiments described here were designed to investigate whether the reported behavioural changes were caused by a direct action of normetanephrine on central structures or were dependent on an increased accumulation of noradrenaline in the brain.[2,3] *In vivo*: Male albino mice (20–25 g) were injected intraperitoneally (i.p.) with pargyline 50 mg/kg. Twenty-four hours later, groups of twelve mice were injected either with normetanephrine HCl dissolved in normal saline or with saline alone. The injections were made into the cerebral ventricles as described by Brittain and Handley,[4] the injection volume in all experiments being 0·02 ml. The mice were normally killed by immersion in liquid nitrogen. While still frozen the brains were removed, weighed in groups of three or six, and pulverised in an anvil. The frozen powder obtained was homogenised in 5·0 ml 0·01N HCl for 90 sec using an M.S.E. high speed rotary cutter (Type 7700) at approximately 12,000 r.p.m. In some cases, animals were killed by dislocation of the neck and subsequent bleeding; in these experiments, brains were weighed in the cold and homogenised immediately.

Noradrenaline was extracted from the homogenates by the method of Shore and Olin,[5] and assayed fluorimetrically using the trihydroxyindole method.[6] Potassium ferricyanide was used as oxidising agent, and the stabilising agent was alkaline ascorbate.

Figure 1 shows the effect of normetanephrine HCl on the brain concentrations of noradrenaline in pargyline-treated mice. The doses of normetanephrine used were between 25 μg and 100 μg and the animals were killed 1 hr after injection. At this time, the animals showed some loss of motor coordination, although this was not quantitatively assessed in these experiments. The difference between noradrenaline levels in treated and control mice is highly significant ($P < 0{\cdot}01$) at all dose levels and there is some evidence of a dose dependent effect.

Addition of normetanephrine HCl to brain extracts from saline-treated control animals showed that, in the doses used, normetanephrine did not interfere with the extraction and assay of noradrenaline.

When the experiments were repeated using mice which had not been pretreated with monoamine oxidase inhibitor, injection of the same doses of normetanephrine HCl, resulted in cerebral noradrenaline levels which were not significantly different from those of saline-injected controls.

Thus these *in vivo* experiments show that normetanephrine will increase the level of noradrenaline in the brain only in animals pretreated with pargyline in a dose sufficient to produce complete inhibition of monoamine oxidase[7] (M.A.O., EC 1.4.3.4). This suggested that normetanephrine might produce its effects on the central nervous system by a process of end product inhibition of catechol-*o*-methyltransferase (C.O.M.T., EC 2.1.1.6), potentiation of the effects of endogenous noradrenaline being produced in a manner analogous to that said to occur at post-ganglionic sympathetic nerve-endings.[2]

To determine whether the observed increase in noradrenaline levels might be the result of C.O.M.T. inhibition by normetanephrine, the following *in vitro* experiments were performed: Catechol-*o*-methyltransferase was prepared from rat liver, as described by Abbs, Broadley and Roberts.[8] Fresh enzyme was prepared every 10 days. Enzyme preparation (1·0 ml) was incubated at 37° with 0·5 ml 0·2 M phosphate buffer pH 8·2, 20 μmoles $MgCl_2$, 20 μmoles methionine HCl, and 20 μ moles ATP in a total volume made up to 2·5 ml with distilled water. *l*-Noradrenaline hydrogen tartrate (1 μmole or 0·5 μ mole) was added to the incubation mixture either alone or ten minutes after the addition of various concentrations of normetanephrine HCl. After 1 hr the reaction was stopped by addition of 0·15 ml 2 N HCl and the reaction mixture was extracted by the method of Shore and Olin[5] at room

temperature. Inhibition of C.O.M.T. was assessed by determination of the residual noradrenaline, using the trihydroxyindole method described above. Using concentrations of normetanephrine HCl ranging from 1 μmole to 100 μmoles, i.e. up to 100 times greater than the substrate concentration, no significant inhibition of C.O.M.T. could be demonstrated.

There is, therefore, no evidence from these experiments that the increased levels of noradrenaline in the brain following injection of normetanephrine are due to the inhibition of C.O.M.T. by a process of end-product accumulation. Alternatively, it might be suggested that at central noradrenergic synapses the local extracellular concentration of noradrenaline may be sufficiently high to involve cellular re-uptake by the process of "Uptake$_2$".[3] It has been shown that this mechanism, which

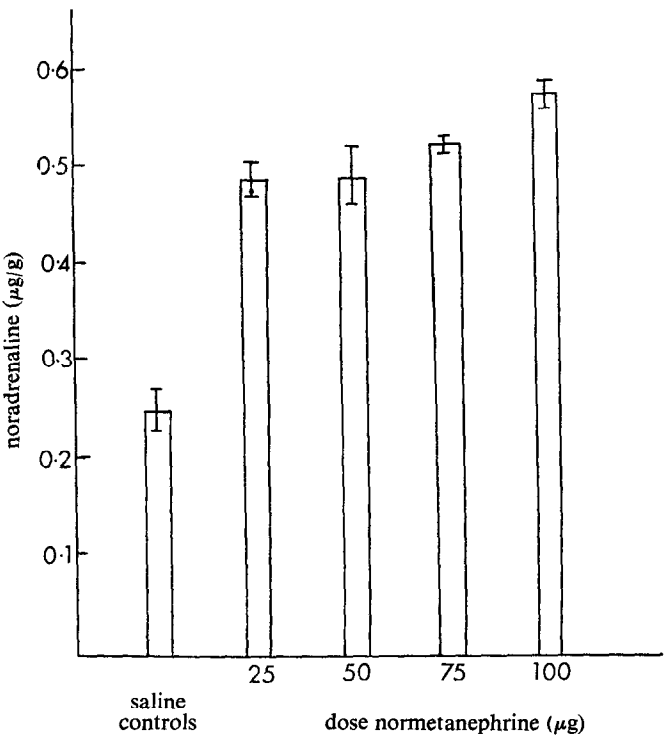

FIG. 1. Effect of normetanephrine on the noradrenaline levels in the brains of mice treated 24 hr previously with pargyline (50 mg/kg, i.p.). Ordinate: brain noradrenaline concentration in μg/g wet wt. Abscissa: Dose of normetanephrine in μg. For each dose level 12 mice (male, albino, 20–25 g) were used. Injections were made into the cerebral ventricles, and the animals were killed 1 hr after injection.

apparently operates only in the presence of high concentrations of amine, is readily inhibited by normetanephrine;[3] such inhibition would clearly tend to increase the extracellular concentration of noradrenaline, although this is apparently not reflected in any change in the overall level of amine in the brain. Potentiation of the behavioural effects of normetanephrine by inhibition of M.A.O.[1] is probably due in part to the inhibition of breakdown of normetanephrine.[8] However the fact that this potentiation is accompanied by an increase in noradrenaline levels, might also suggest that inhibition of uptake by normetanephrine produces an increase in extracellular noradrenaline sufficient to saturate available C.O.M.T. The simultaneous inhibition of M.A.O. would thus lead to a further accumulation of noradrenaline, as reported here.

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Typical hyperaminoaciduria after high doses of 6-azauridine triacetate

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THE BIOCHEMICAL effect of 6-azauridine triacetate (6-AzUR-TA) consists of an interference with the biosynthesis of the pyrimidine nucleotide components of the nucleic acids. The site of blockade is the enzyme which decarboxylates orotidine 5′-phosphate; to accomplish this 6-AzUR-TA is converted to 6-azauridine 5′-phosphate, which is the specific inhibitor of orotidine 5′-phosphate decarboxylase. Large amounts of orotic acid and orotidine excreted in urine of animals and patients after application of 6-azauridine seem to be caused by this blockade.

However, Bono *et al.*[1] found, using ^{14}C-orotic acid administered intravenously, that patients treated with 6-azauridine have in some instances an increased production of uridine 5′-phosphate. Accumulation of uridine 5′-phosphate in patients treated with 6-azauridine might result in an excretion of β-alanine as the degradative product of uracil formed from uridine 5′phosphate. Also, carbamyl aspartic acid, found in an increased amount in the urine of patients treated with 6-azauridine,[1] could be considered as the source of β-alanine.[2] Furthermore, it was possible that a relationship existed between these changes and the metabolism of certain amino acids. These questions were considered to be important in view of the clinical application of 6-AzUR-TA for the treatment of tumors,[3, 4, 5] viral infections,[6] *mycosis fungoides*[7] and psoriasis.[8, 9]

Four patients aged 43–53, suffering from a generalized form of psoriasis, were treated *per os* with 400 mg of 6-AzUR-TA per kg of body weight per day for 3 weeks. The amino acids in blood and urine were isolated quantitatively by two-dimensional paper chromatography after dilution and filtration of the sample through a column of Dowex-50 × 8 according to the method of Hyánek[10] and detected with ninhydrin. The determinations were carried out in duplicate from a 24-hr sample of urine before and after acid hydrolysis. β-Alanine was identified by paper chromatography by using a known compound as a standard in various elution systems: *n*-butanol: acetic acid: water