drenaline and 5-hydroxytryptamine concentrations were determined by the methods of Welch & Welch (1969) and Curzon & Green (1970) respectively. When compared with litter-mate untreated rats, noradrenaline concentrations were found to have been reduced in all areas of the brain by chronic methylamphetamine administration; 5-hydroxytryptamine concentrations were also reduced in the striatum and mid-brain, but were unaltered in the thalamus/hypothalamus, the hippocampus and the pons/medulla, and were increased in the cortex. In all areas except the striatum, 5-hydroxytryptamine concentrations were lower 24 than 0 hours after withdrawal. Noradrenaline concentrations fell steadily for 36 h in the striatum, rose, for 48 h in the thalamus/hypothalamus and cortex, and showed an initial rise in the hippocampus, mid-brain and pons/medulla followed in the cases of the mid-brain and pons/medulla by falls to below 0 h levels 24 h after withdrawal.

These results suggest that chronic methylamphetamine ingestion may affect not only the release of monoamines, but also their synthesis and transport along neuron axons. They also suggest a biochemical basis for the marked "post-amphetamine depression" frequently reported in man.

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Biogenic amines and the anti-nociceptive activity of agents with a non-opiate structure

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The anti-nociceptive activity of morphine has been associated with several putative central neurotransmitters, including 5-hydroxytryptamine (5HT) and noradrenaline (NA). The interactions of these amines with morphine have been studied using intracerebroventricular (ICV) injections both in rats (Sparkes & Spencer, 1971) and mice (Calcutt & Spencer, 1971). A recent report from our laboratory (Sewell & Spencer, 1974) showed that in mice, ICV-administered 5-HT acutely potentiated not only morphine's anti-nociceptive activity, but also that of a range of narcotic agonist and partial agonist agents, whilst ICV-administered NA antagonized the effects of these agents. Each of the agents examined so far has been structurally related to the naturally-occurring opiates, and the purpose of this report is to describe the effects of these amines when given to animals receiving analgesics of clearly different chemical structure.

Male albino mice of the ICI strain, weighing 18–22 g, were used throughout, and nociceptive sensitivity was determined using the tail immersion technique. Four analgesics were examined: pethidine; AH 7921, 3,4-dichloro-*N*-(1-dimethylamino) cyclohexylmethyl benzamide, a recent analgesic shown to be active in the mouse, dog and monkey (Brittain, Kellett & others, 1973); profadol, *m*-(1-methyl-3-pyrrodinyl) phenol, which is active clinically (Beaver, Wallenstein & others, 1969); and (+)-amphetamine. All four possess activity in this test, but with different slopes to their dose-response lines, AH 7921 possessing the steepest and (±)-amphetamine the shallowest slopes. ICV-administered 5-HT (10 μg/animal) significantly prolonged the anti-nociceptive effect of pethidine (15 mg kg^{-1}), AH 7921 (2·5) and profadol (10). Further, as previously reported with the opiates, ICV NA (10 μg) significantly attenuated the activity of pethidine (50 mg kg^{-1}), AH 7921 (5) and profadol In contrast, ICV-administered 5HT (10 μg) significantly reduced the activity of (±)-amphetamine (5 mg kg^{-1}), whilst NA (10 μg) marginally potentiated the effects of (+)-amphetamine (7·5).

As well as pethidine, AH7921 and profadol have each previously been classified as narcotic analgesics. Consequently it appears that, irrespective of chemical structure, narcotic agonist and partial-agonist analgesics will be potentiated by ICV-administered 5HT and antagonized by NA, whilst agents with a different anti-nociceptive action will interact with

these two amines in a qualitatively different fashion. It is thus possible that the type of interaction between these two amines and an analgesic might be a useful acute method of predicting whether or not the analgesic is narcotic in character.

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The binding of zinc to human serum proteins

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Zinc has been shown to be bound by the proteins in human serum with 2–8% being associated with low molecular weight components (Prasad & Oberleas, 1970). As previous work has been with ionic zinc, we have studied the binding of zinc from a zinc aminoacid complex.

^{65}Zn (L-Histidine)$_2$2H$_2$O was prepared and volumes (0·1 to 2·0 ml) of a standard solution (1·75 mg Zn per ml and specific activity 34 μ Ci ml^{-1}) were mixed with constant volumes (5·0 ml) of pooled human serum and the mixture shaken at 37° for 30 min. The percentage of zinc associated with low molecular weight constituents of serum was found by separating the protein bound zinc in a "Millipore" hi-flux ultrafiltration cell having a "Pellicon" membrane of nominal molecular weight cut off 1000. The ultra-filtrate (0·1 ml) was examined for ^{65}Zn activity using a Harwell model 2000 well type scintillation counter. The activity in the ultrafiltrate was expressed as a percentage of the initial activity in the mixture prior to ultrafiltration.

The results were compared with those obtained using a standard solution of $^{65}ZnCl_2$ (1·75 mg Zn per ml and specific activity 135 μ Ci per ml) in similar experiments and shown in Fig. 1.

When the volume of $^{65}ZnCl_2$ solution added ranged from 0·1 ml to 1·0 ml the percentage of ^{65}Zn appearing in the ultrafiltrate was of the order of 2–3%. As the volume of ^{65}Zn Cl_2 solution was increased above 1·0 ml a sharp increase in the percentage of ^{65}Zn in the ultrafiltrate occurred until 9% appeared after 2·0 ml had been added. In the case of ^{65}Zn (L-His)$_2$.2H$_2$O the amount of ^{65}Zn appearing in the ultrafiltrate was greater by a factor of 10; from 10% for 0·1 ml of the standard solution added to 90% for 2·0 ml. Also the shape of the plot indicates that there is an exponential relationship between the amount of ^{65}Zn added and the amount of "free" zinc, i.e. zinc not bound by serum proteins.

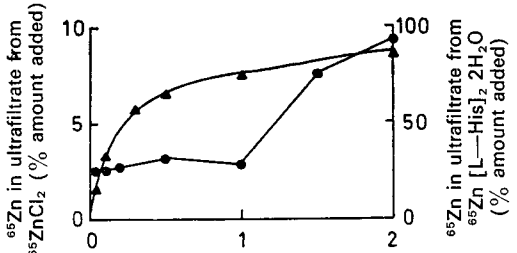

FIG. 1. The percent of ultrafiltrable ^{65}Zinc plotted against the volume of ^{65}Zinc solutions added. ▲——▲——▲ Zn (His)$_2$ 2H$_2$O: ●——●——● $ZnCl_2$.