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# The reversal of the central effects of noradrenaline by antidepressant drugs in mice

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1. Noradrenaline given directly into the lateral cerebral ventricles induced hypothermia in mice. This hypothermia was antagonized and eventually reversed to a hyperthermia by imipramine-like antidepressant drugs.

2. The mechanism of action involved in this effect of antidepressant drugs has been studied using nortriptyline as a typical representative of antidepressant drugs.

3. Nortriptyline pretreatment did not modify either the uptake, subcellular distribution, or the metabolism of $^3$H-noradrenaline injected into the lateral cerebral ventricles.

4. Nortriptyline had the same order of activity in reversing the hypothermia produced by the intraventricular injection of noradrenaline irrespective of whether it was given directly into the lateral cerebral ventricles or subcutaneously.

5. Noradrenaline given subcutaneously caused hyperthermia in mice which antagonized and reversed the hypothermia induced by noradrenaline given directly into the lateral ventricles.

6. The antagonism by both noradrenaline given subcutaneously and nortriptyline was reduced to the same degree by $\alpha$- and $\beta$-adrenoceptive receptor blocking agents.

7. Nortriptyline, at dose levels required to antagonize and reverse the hypothermia induced by intraventricular injections of noradrenaline, potentiated the hyperthermia caused by noradrenaline given subcutaneously in conscious mice and the pressor responses to noradrenaline given either intravenously or into the lateral ventricles in anaesthetized mice.

8. It is suggested that imipramine-like antidepressant drugs antagonize the hypothermia produced by intraventricular injections of noradrenaline by potentiating the hyperthermic effects of that part of the centrally administered noradrenaline that passes to the periphery rather than a direct central antagonism of the effects of noradrenaline.

Brittain (1966) reported that pretreatment with imipramine-like antidepressant drugs antagonized the depressive and hypothermic actions of noradrenaline injected into the lateral cerebral ventricles in mice. In view of the well-documented potentiation of the effects of noradrenaline which these compounds produce peripherally, this apparent central antagonism of the effects of noradrenaline is paradoxical.

The data reported in this paper suggest that this antagonism results from a potentiation of the hyperthermic effects of that portion of the noradrenaline administered into the lateral ventrical which passes to the periphery rather than a direct central antagonism of the hypothermic action of noradrenaline. A brief account of these experiments was presented to the British Pharmacological Society (Cowell & Davey, 1968).

## Methods

Oesophageal temperatures of conscious male albino mice of the *T.O.* strain weighing 18–20 g were recorded with a thermocouple (Brittain & Spencer, 1964). All experiments were performed at an ambient temperature of 19° C.

Compounds were introduced directly into the lateral cerebral ventricles of mice following the technique described by Haley & McCormick (1957) using a 3 mm long 25 gauge syringe needle attached to a microsyringe. Compounds were dissolved in 0.9% w/v sodium chloride, and the total volume administered never exceeded 0.02 ml. Ascorbic acid (0.002% w/v) was included in each dilution of noradrenaline bitartrate.

That this technique provided a reliable means of introducing compounds directly into the lateral cerebral ventricles was confirmed by ventriculography in six mice using 0.01 ml. of iophendylate injection (Myodil) as the contrast medium.

In experiments with anaesthetized mice, sodium pentobarbitone (60 mg/kg) given intraperitoneally was used as the anaesthetic. Arterial pressure was recorded from a cannulated carotid artery using a pressure transducer and Devices recorder. Drugs were administered intravenously via a needle inserted in a tail vein.

### *Subcellular distribution of $^3$H-noradrenaline*

Six mice were given nortriptyline (10 mg/kg) orally 1 and 4 hr before 10 $\mu$g DL-noradrenaline hydrochloride 7-$^3$H ($10^5$ d.p.m./$\mu$g) given into the lateral ventricle. A further six mice served as controls, being given an equivalent volume of 0.9% w/v NaCl intraventricularly.

The mice were killed by cervical dislocation 15 min after the $^3$H-noradrenaline, and the brains of three mice pooled to provide two treated and two control brain samples.

The brain samples were homogenized in 9 vol. of 0.25 M sucrose at 4° C. The total particulate material was obtained by centrifugation at 39,000 rev/min for 1 hr at 4° C in a Spinco No. 40 angle rotor. The particulate material was resuspended in 0.25 M sucrose. The total radioactivity of the samples was obtained by dissolving aliquots of both the supernatant and particulate fractions in N.C.S. reagent (Nuclear Chicago), to which toluene scintillator was added and counted in a Nuclear Chicago liquid scintillation counter. Counting efficiencies were determined using an external $^{133}$Ba source.

Aliquots of the supernatant and particulate fractions were extracted with 0.3 M perchloric acid ; catechols were separated from *o*-methylated metabolites by adsorption on an alumina column and elution with 0.2 M acetic acid (Merrills, 1963). Dioxan scintillator was added to samples of the column fractions for measurement of radioactivity as above.

### *Drugs*

Drugs used were: L-noradrenaline bitartrate (Upjohn); nortriptyline (Lilly Research Laboratories Ltd.); pentobarbitone sodium (Nembutal, Abbott Laboratories Ltd.); propranolol (Inderal, I.C.I.); dibenamine (L. Light and Co. Ltd.); tolazoline (Priscol, Ciba Laboratories Ltd.); phentolamine (Rogitine, Ciba Laboratories Ltd.); phenoxybenzamine (Dibenzyline, Smith, Kline and French); pempidine tartrate (I.C.I.); iophendylate (Myodil, Glaxo Laboratories Ltd.); imipramine (Tofranil, Geigy U.K. Ltd.); desmethylimipramine (Pertofran, Geigy U.K. Ltd.); amitriptyline (W. R. Warner & Co. Ltd.); DL-7-$^3$H noradrenaline hydrochloride, $10^{-5}$ d.p.m./$\mu$g (Amersham Radiochemicals).

## Results

### *Effect of nortriptyline on the hypothermia induced by noradrenaline given intraventricularly*

The effects of nortriptyline (0.5, 1.0 and 5 mg/kg) given subcutaneously 60 min before the injection of noradrenaline (10 $\mu$g) into the lateral ventricle in mice are shown in Fig. 1. The hypothermia produced by noradrenaline was antagonized by small amounts of nortriptyline and reversed to a hyperthermia by larger amounts of nortriptyline, thus confirming the original results of Brittain (1966).

### *Effects of nortriptyline on uptake, metabolism and subcellular distribution of $^3$H-DL-noradrenaline injected into the lateral ventricle*

An attempt was made to correlate the reversal by nortriptyline of the hypothermia produced by injection of noradrenaline into the lateral cerebral ventricles with changes in either the uptake, metabolism or the subcellular distribution of noradrenaline. In a preliminary experiment, nortriptyline (10 mg/kg) was given orally 1 and 4 hr before the intraventricular injection of $^3$H-DL-noradrenaline hydrochloride $10^5$ d.p.m./$\mu$g (10 $\mu$) (Table 1). Pretreatment with nortriptyline prevented the hypothermia but failed to modify appreciably either the uptake, metabolism or subcellular distribution of $^3$H-noradrenaline. This approach was therefore discontinued and investigations directed towards possible peripheral mechanisms of action for nortriptyline.

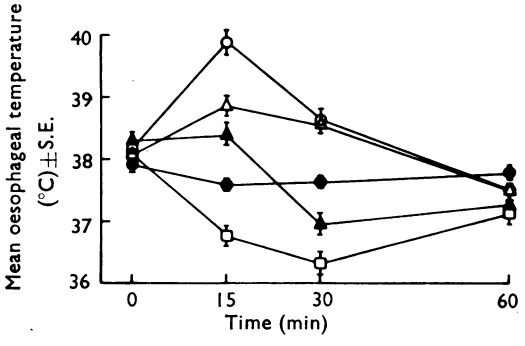


FIG. 1. Record of the hypothermia produced by noradrenaline (10 $\mu$g) injected into the lateral cerebral ventricles of conscious mice and the antagonism and eventual reversal of this response by nortriptyline (0.5, 1.0 and 5.0 mg/kg) given subcutaneously 60 min before the intraventricular injection of noradrenaline. Room temperature 19° C. ●——●, Saline ($n$=40); □——□, noradrenaline (10 $\mu$g) ($n$=40), ▲——▲, nortriptyline (0.5 mg/kg) ($n$=38); △——△, nortriptyline (1.0 mg/kg) ($n$=40); ○——○, nortriptyline (5.0 mg/kg) ($n$=15).

TABLE 1. *Effect of nortriptyline on uptake, metabolism and subcellular distribution of centrally administered* $^3$*H-*DL*-noradrenaline in mouse brain* in vivo

| Pretreatment | Lat. vent. | % uptake 15 min | % radioactivity in particulate fraction | | | % radioactivity in supernatant fraction | | | Oesophageal temperature (°C) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total | % of total | | Total | % of total | | Time 0 | 15 min |
| | | | | *o*-methylated metabolites | Catechols | | *o*-methylated metabolites | Catechols | | |
| Controls: saline | $^3$H-DL-noradrenaline ($10^5$ d.p.m./$\mu$g) 10 $\mu$g | 26 | 41 | 26 | 72 | 60 | 29 | 72 | 38·29 ±0·18 | 35·16 ±0·18 |
| Nortriptyline 10 mg/kg p.o. 1 and 4 hr | $^3$H DL-noradrenaline ($10^5$ d.p.m./$\mu$g) 10 $\mu$g | 24 | 39 | 30 | 67 | 63 | 33 | 66 | 38·65 ±0·31 | 39·35 ±0·53 |

*Antagonism of the hypothermia induced by intraventricular administration of noradrenaline by nortriptyline given either intraventricularly or subcutaneously*

The antagonistic activity of nortriptyline (0.125, 0.25, 0.5 and 1.0 mg/kg) was of the same order against the hypothermia produced by the intraventricular injection of noradrenaline (10 $\mu$g), irrespective of whether nortriptyline was injected either directly into the lateral ventricle or subcutaneously 30 min before the injection of noradrenaline into the lateral ventricle (Fig. 2). Although nortriptyline (0.25, 0.5 and 1.0 mg/kg) was statistically significantly more active when injected directly into the lateral cerebral ventricle, this difference was not of the same order as would be anticipated for a direct central antagonism of noradrenaline by nortriptyline.

It was therefore decided to investigate the possibility that this antagonistic action of nortriptyline was a consequence of a peripheral effect.

*Effect of nortriptyline on the hyperthermia induced by noradrenaline administered subcutaneously*

Noradrenaline (0.5–4.0 mg/kg) given subcutaneously caused a marked hyperthermia in mice. This hyperthermia was potentiated by imipramine (10 mg/kg), desmethylimipramine (10 mg/kg), amitriptyline (10 mg/kg) and nortriptyline (10 mg/kg) given orally 1 hr before the subcutaneous injection of noradrenaline (0.5 mg/kg) (Fig. 3). This is in agreement with the results of Jori, Paglialunga & Garattini (1967) in rats.

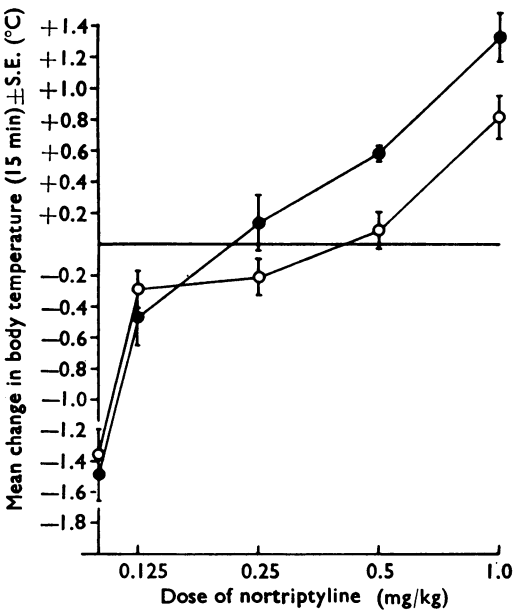


FIG. 2. Record of the antagonism and reversal of the hypothermia produced by the injection of noradrenaline (10 $\mu$g) into the lateral ventricle by nortriptyline (0.125, 0.25, 0.5 and 1.0 mg/kg; $n=40$ at each dose level) given either directly into the lateral cerebral ventricle (●) or subcutaneously (○) 30 min before the intraventricular injection of noradrenaline. Room temperature 19° C.

Nortriptyline (0.125–1.0 mg) given either subcutaneously or intraventricularly 30 min before the subcutaneous injection of noradrenaline (0.5 mg/kg) produced a dose-related potentiation of the hyperthermia (Fig. 4). These amounts of nortriptyline were the same as those required to antagonize and eventually to reverse the hypothermia induced by noradrenaline given into the lateral ventricle (Fig. 2).

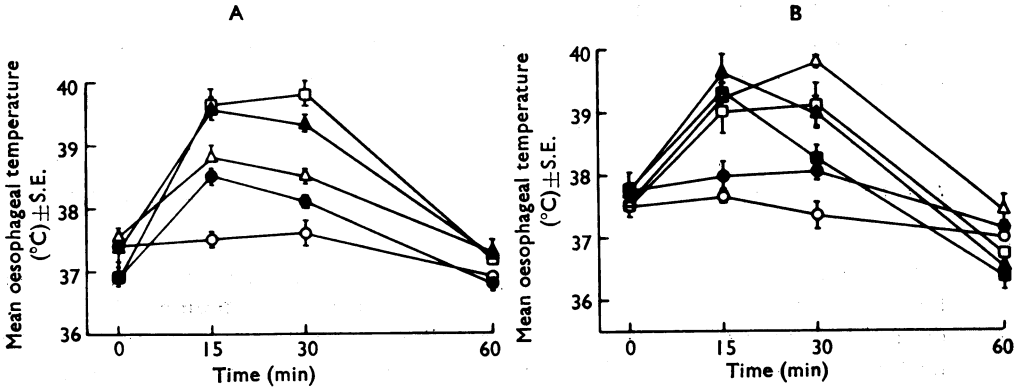


FIG. 3. A: Record of the hyperthermia produced by the subcutaneous injection of noradrenaline 0.5 (●), 1.0 (△), 2.0 (▲) and 4.0 (□) mg/kg given in conscious mice. (○), Control (saline). B: The potentiation of the hyperthermia produced by subcutaneously administered noradrenaline (0.5 mg/kg) (●), and by nortriptyline (△), imipramine (▲), desmethylimipramine (□) and amitriptyline (■), all given at a dose level of 10 mg/kg orally 60 min before the injection of noradrenaline. (○), Control (saline). Room temperature 19° C. $n=10$ in each group.

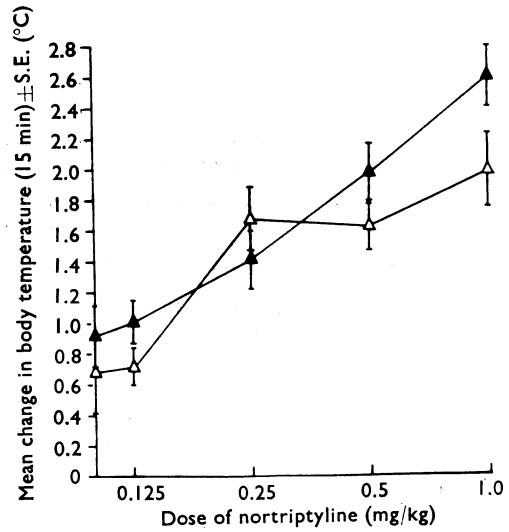


FIG. 4. Record of the potentiation of the hyperthermia produced by subcutaneously administered noradrenaline (0.5 mg/kg) by nortriptyline (0.125, 0.25, 0.5 and 1.0 mg/kg) given either intravenously (▲) or subcutaneously (△) 30 min before the noradrenaline. $n=20$ at each dose level. Room temperature 19° C.

*Effect of subcutaneously administered noradrenaline on the hypothermic response to noradrenaline given into the lateral ventricle*

The simultaneous subcutaneous injection of noradrenaline (0.25, 0.5, 1.0 and 2.0 mg/kg) antagonized and subsequently reversed the hypothermia evoked by the intraventricular injection of noradrenaline 10 $\mu$g (Fig. 5). Thus noradrenaline given subcutaneously modified the hypothermia induced by injection of noradrenaline into the lateral ventricle in an identical manner to nortriptyline and other antidepressant compounds. This finding was compatible with the view that the antagonism by antidepressant drugs resulted from the potentiation of the peripheral effects of noradrenaline.

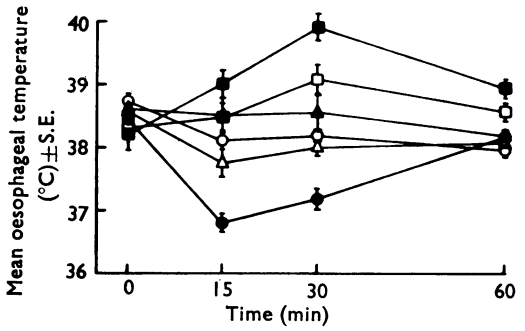


FIG. 5. Record of the antagonism and reversal of the hypothermia evoked by the intraventricular injection of noradrenaline (10 $\mu$g) (●) by the simultaneous subcutaneous injection of noradrenaline 0.25 (△), 0.5 (▲), 1.0 (□) and 2.0 (■) mg/kg. (○), Control (saline). $n=10$ in each group. Room temperature 19° C.

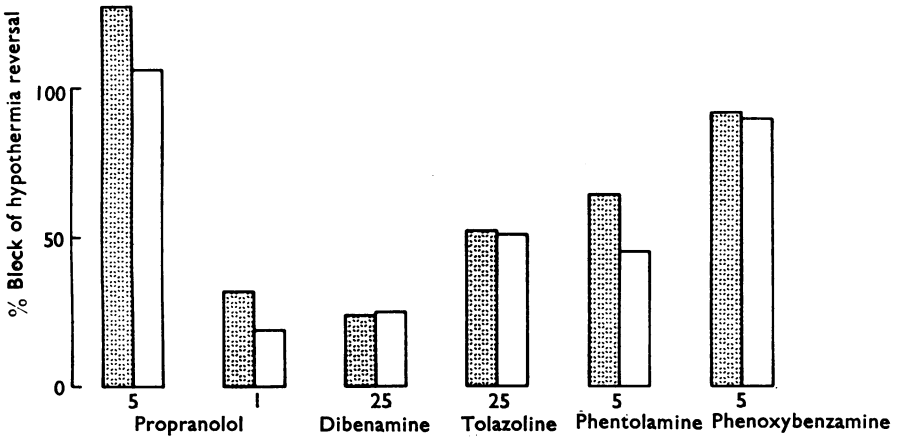


FIG. 6. Histogram of the average percentage reduction by propranolol (1 and 5 mg/kg), dibenamine (25 mg/kg), tolazoline (25 mg/kg), phentolamine (5 mg/kg) and phenoxybenzamine (5 mg/kg) of the reversal of the centrally induced noradrenaline (10 $\mu$g) hypothermia by nortriptyline (10 mg/kg) given orally (□) and noradrenaline (1 mg/kg) given subcutaneously (▦). All compounds were administered 60 min before the intraventricular injection of noradrenaline, with the exception of the subcutaneous injection of noradrenaline, which was given simultaneously. $n=20$ in each group. Room temperature 19° C.

*Effect of α- and β-adrenoceptive receptor blocking drugs on reversal of central noradrenaline hypothermia by subcutaneous noradrenaline and nortriptyline*

The α-adrenoceptive receptor blocking drugs, phenoxybenzamine (5 mg/kg), phentolamine (5 mg/kg), tolazoline (25 mg/kg), dibenamine (25 mg/kg) and the β-adrenoceptive receptor blocking drug propranolol (1 and 5 mg/kg) antagonized to a similar extent the reversal of noradrenaline hypothermia by both nortriptyline (10 mg/kg) and the subcutaneous injection of noradrenaline (1 mg/kg) (Fig. 6). This observation suggests a common mode of action and further strengthens the possibility that the antagonism of the hypothermia caused by noradrenaline given into the lateral ventricle may be the result of the peripheral blockade of the uptake of noradrenaline by nortriptyline and other antidepressant drugs.

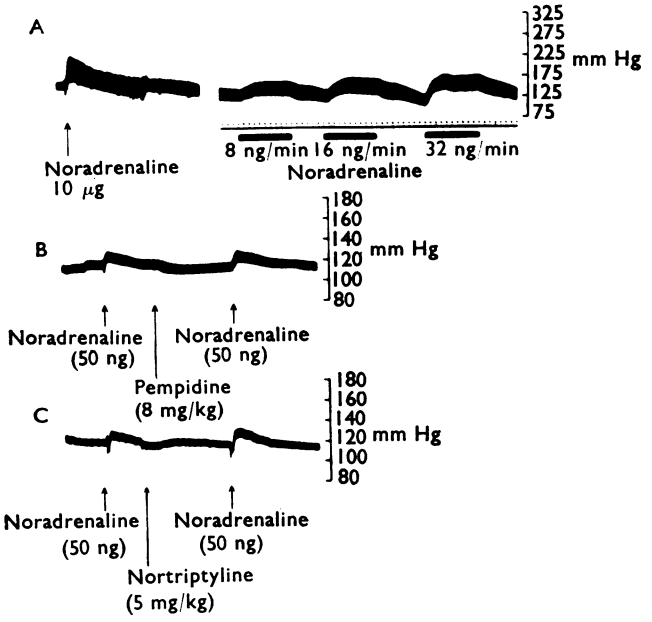


FIG. 7. Records of mouse arterial blood pressure. Pentobarbitone sodium anaesthesia. A: comparison of the pressor response to noradrenaline (10 μg) injected into the lateral cerebral ventricle with those to intravenous infusions of noradrenaline (8, 16 and 32 ng/min) in the same mouse. Time scale 1 min. B: Slight potentiation by pempidine (8 mg/kg) of the pressor effects of noradrenaline (50 ng) given intraventricularly. C: Potentiation of the pressor response to noradrenaline (50 ng) given intraventricularly by nortriptyline (5 mg/kg) given subcutaneously.

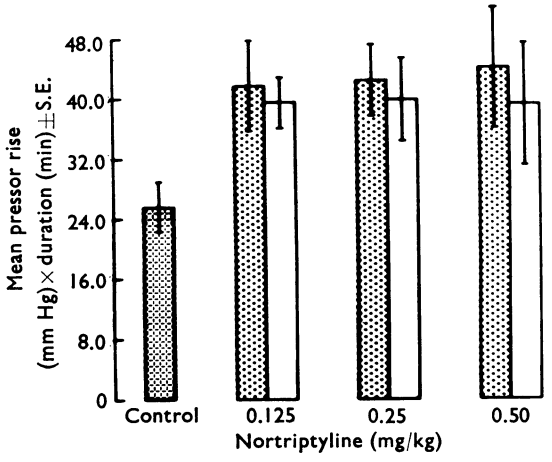


FIG. 8. Histogram showing the potentiation of the pressor responses to noradrenaline (2 ng), given intravenously, by nortriptyline (0.125, 0.25 and 0.50 mg/kg) given either subcutaneously (□) or intraventricularly (▦) in mice anaesthetized with sodium pentobarbitone. $n = 10$ in each group.

*Blood pressure responses to noradrenaline given into the lateral ventricle*

Noradrenaline (10 $\mu$g) injected directly into the lateral cerebral ventricles caused an increase in arterial pressure of approximately 75 mm Hg and 15 min duration in mice anaesthetized with sodium pentobarbitone (Fig. 7). This pressor response was similar in magnitude to that produced by a constant intravenous infusion of noradrenaline (160 ng) at a rate of 16 ng/min (Fig. 7). The pressor response to noradrenaline (50 ng) given into the lateral ventricle was not reduced by doses of pempidine sufficient to abolish the pressor response to dimethylphenylpiperazinium. Thus the pressor response was not the result of a central increase in efferent sympathetic discharge but was presumably due to the leakage to the periphery of part of the noradrenaline given into the lateral cerebral ventricle. Furthermore, this pressor response was potentiated by nortriptyline (5 mg/kg) given subcutaneously (Fig. 7).

*Effect of nortriptyline on pressor responses to intravenously administered noradrenaline in the anaesthetized mouse*

Nortriptyline (0.125, 0.25 and 0.50 mg/kg) given either subcutaneously or intraventricularly potentiated the pressor responses to intravenous injections of noradrenaline in mice anaesthetized with sodium pentobarbitone (Fig. 8). These were the same amounts of nortriptyline that were required both subcutaneously and intraventricularly to antagonize and reverse the hypothermia induced by intraventricular injection of noradrenaline (Fig. 2).

## Discussion

The reported finding of Brittain (1966) that antidepressant drugs antagonized the depressive and hypothermic actions of noradrenaline injected into the lateral ventricles in mice appeared contradictory to the current concept that these agents facilitate adrenergic mechanisms.

Pretreatment with nortriptyline prevented the hypothermia confirming the results of Brittain (1966), but failed to modify significantly either the uptake, metabolism or subcellular distribution of $^3$H-noradrenaline. This finding, together with the fact that the antagonistic potency of nortriptyline administered directly into the lateral ventricle was of the same order as that obtained following peripheral administration, in our opinion mitigated against a central action of nortriptyline in this situation.

Noradrenaline given subcutaneously caused a hyperthermia in mice which was markedly potentiated by imipramine-like antidepressant compounds. This finding suggested that the antagonistic action of imipramine-like compounds was a consequence of their peripheral effects rather than a direct central antagonism of noradrenaline. This possibility was further strengthened by the fact that both the reversal of the hypothermia by noradrenaline given subcutaneously and by imipramine-like antidepressant drugs were antagonized to the same degree by $\alpha$- and $\beta$-adrenoceptive receptor blocking agents.

Noradrenaline given directly into the lateral cerebral ventricle caused a pressor response in anaesthetized mice which was potentiated by pempidine, indicating that this pressor response was not the result of a centrally induced increase in sympathetic activity. In addition, nortriptyline given either directly into the lateral

ventricle or subcutaneously, markedly potentiated the pressor responses to noradrenaline also given directly into the lateral ventricle or subcutaneously in anaesthetized mice, at the dose levels required to antagonize the centrally induced hypothermic action of noradrenaline in conscious mice.

On the basis of these results it is suggested that after an injection of noradrenaline into the lateral ventricle, a balance results between the central hypothermic effect of noradrenaline, and the hyperthermic effect of the portion which escapes to the peripheral circulation. This balance under normal conditions falls in favour of the hypothermia. In the presence of antidepressant drugs, however, the effects of peripheral circulating noradrenaline are enhanced, and the balance then swings in favour of the peripheral actions of noradrenaline, the degree of swing being dependent on the dose of antidepressant drug, the maximal effect being shown by a complete reversal to a hyperthermia.

This hypothesis explains not only why imipramine-like antidepressant drugs antagonize the central hypothermic effects of noradrenaline, but also why a reversal to a hyperthermia occurs. It is also consistent with the known pharmacological properties of imipramine-like antidepressant drugs—namely, their well-documented effects in blocking the uptake of noradrenaline.

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