Br. J. Pharmac. (1969), **36**, 85–96.

The interactions of noradnamine and imipramine-like antidepressant drugs

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1. The hypothesis of Roberts & Broadley (1965) that noradnamine formation in the brain is responsible for endogenous depression has been investigated in mice.

2. Injections of noradnamine given directly into the lateral ventricles caused convulsions and profound hypothermia, but were without effect if given subcutaneously.

3. The hypothermia, but not the convulsions, induced by noradnamine was reversed by imipramine-like antidepressant drugs given before or after the injection of noradnamine. The convulsions but not the hypothermia were abolished by phenobarbitone.

4. Increasing doses of nortriptyline produced a parallel shift of the hypothermic log dose-response curve for intraventricular injections of noradnamine to the right.

5 The minimal effective dose of nortriptyline required to reverse noradnamine hypothermia was the same whether the nortriptyline was injected directly into the lateral ventricle or subcutaneously.

6. No evidence was found to substantiate the claim that reserpine hypothermia is mediated by noradnamine formation in the brain.

7. Intraventricular, but not intraperitoneal, injection of noradnamine caused a depletion of brain noradrenaline and an increase in brain 5-hydroxytryptamine. These changes did not result from the convulsive activity and were not modified by pretreatment with nortriptyline. No effect on heart noradrenaline levels was recorded.

8. Noradrenaline, given subcutaneously, also antagonized the hypothermic response to noradnamine.

9. The reversal of noradnamine hypothermia by both noradrenaline given subcutaneously and nortriptyline was blocked by α and β-adrenoceptive receptor blocking agents.

10. It is considered that the mode of action of the antagonism of noradnamine hypothermia by imipramine-like antidepressant drugs is a peripheral and not a central mechanism and probably results from a potentiation of the effects of circulating noradrenaline released by noradnamine.

Roberts & Broadley (1965) confirmed and extended the observation of Kawazu (1958) that adrenaline and noradrenaline formed 5-methylaminomethyl and 5-amino-

methyl, 2,3,7,8-tetrahydroxydibenzo-(a-e) cycloheptatriene (adnamine and noradnamine) respectively in the presence of certain mineral acids.

Roberts & Broadley (1965) were sufficiently impressed by the close structural resemblance of these compounds to imipramine-like antidepressant drugs that they postulated that noradnamine was probably an anabolic product of noradrenaline. The authors suggested that an imbalance between catechol-O-methyl transferase and monoamine oxidase in the sense of decreased activity of the former and increased activity of the latter would lead to an excessive formation of noradnamine. The increased noradnamine levels in the brain would then be responsible for the development of endogenous depression. The imipramine-like antidepressant drugs were considered to be competitive antagonists of noradnamine and the antidepressive activity of monoamine oxidase inhibitors considered to be the consequence of an inhibition of the synthesis of noradnamine from noradrenaline.

This concept would also offer an explanation for the adverse depressant action of reserpine in man. Since the monoamine oxidase present in the mitochondria of adrenergic nerves competes with the ATP–Mg^{++} dependent incorporation process for cytoplasmic noradrenaline, it would follow that when the latter was specifically blocked by low concentrations of reserpine, the synthesis of noradnamine by monoamine oxidase would be facilitated.

As the search for new drugs for the treatment of endogenous depression is handicapped by the absence of decisive knowledge about the aetiology of endogenous depression, it seemed relevant to test the validity of this hypothesis.

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 following the technique described by Haley & McCormick (1957) using a 3 mm long 25 gauge hypodermic 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. That this technique provided a reliable means of injecting compounds directly into the lateral cerebral ventricles was confirmed in six mice using 0·01 ml. of iophendylate injection (Myodil) with subsequent *x*-ray examination (Cowell & Davey, 1968).

The 5-hydroxytryptamine and noradrenaline content of mouse brains were determined by the methods of Kuntzman, Shore, Bogdanski & Brodie (1961) and Merrills (1963) respectively.

The effects of drugs on the hypothermia induced by prior treatment with reserpine were investigated using the methods of Askew (1963). The oesophageal temperatures of the mice were measured at 60 min intervals after intraperitoneal injection of the test drug for a total period of 5 hr. A 0·25% w/v stock solution of reserpine was prepared by dissolving reserpine in a minimal quantity of glacial acetic acid, followed by the addition of propylene glycol (15% v/v), absolute ethanol (5% v/v) and distilled water to 100%. This stock solution was diluted 1 to 10 with distilled water before use and was injected subcutaneously in a volume of 10 ml./kg (2·5 mg/kg).

Noradnamine was prepared by the method of Kawazu (1958). The product was shown to be free from noradrenaline by paper chromatography using an acetic acid/butanol/water (1 : 4 : 5) system.

Drugs for injection were dissolved in 0·9% w/v sodium chloride with the addition of 0·002% w/v ascorbic acid for solutions of noradnamine and noradrenaline bitartrate.

The following drugs were used: (−)-noradrenaline bitartrate (Upjohn). Nortriptyline (Lilly Research Laboratories Ltd.). Propranolol (Inderal, I.C.I.). Tolazoline (Priscol, Ciba Laboratories Ltd.). Phenoxybenzamine (Dibenzyline, Smith, Kline & French Laboratories Ltd.). Iophendylate (Myodil, Glaxo Laboratories Ltd.). Imipramine (Tofranil, Geigy, U.K. Ltd.). Desmethylimipramine (Pertofran, Geigy U.K. Ltd.). Amitriptyline (W. R. Warner & Co. Ltd.). Reserpine B.P. (Courtin & Warner). (±)-amphetamine sulphate B.P. (May & Baker Ltd.). Methylphenidate (Ritalin, Ciba Laboratories Ltd.). Cocaine hydrochloride (May & Baker Ltd.). Nialamide (Niamid, Pfizer Ltd.). Tranylcypromine sulphate (Parnate, Smith, Kline & French Laboratories Ltd.). Tripelennamine hydrochloride (Pyribenzamine, Ciba Laboratories Ltd.). Phenindamine tartrate (Thephorin, Roche Products Ltd.). Diphenhydramine hydrochloride (Benadryl, Parke, Davis & Co.). Chlordiazepoxide hydrochloride (Librium, Roche Products Ltd.). Chlorpromazine hydrochloride (Largactil, May & Baker Ltd.). Pargyline hydrochloride (Eutonyl, Abbott Laboratories Ltd.). Promethazine hydrochloride (Phenergan, May & Baker Ltd.). Atropine sulphate (The British Drug Houses Ltd.). Mepyramine maleate (Anthisan, May & Baker Ltd.).

Results

Noradnamine—symptomatology in mice

No effects were seen in mice after noradnamine (10 mg/kg) given subcutaneously. Noradnamine (10–40 μg) injected directly into the lateral ventricles of mice caused clonic convulsions accompanied by rolling, reduced motor activity, circling behaviour and scratching 1·5–2 hr after dosing. Intraventricular injections of noradnamine up to 20 μg were not lethal. Noradnamine (40 μg), however, caused the death of seven out of ten mice within 5 hr.

Effect of noradnamine on body temperature of mice

Noradnamine (10, 20 and 40 μg) injected directly into the lateral ventricles caused a profound hypothermia (Fig. 1). Noradnamine (10 μg) caused a fall in oesophageal temperature of 4 hr duration, the maximum effect (2° C) occurring 2 hr after administration of the compound. Noradnamine (20 μg) caused a pronounced and prolonged fall of 6° C for more than 12 hr but less than 24 hr duration. An intraventricular injection of noradnamine (40 μg) produced a similar response. As mentioned above, however, seven out of ten mice died within 5 hr. Noradnamine (5 μg) caused only transient hypothermia. These results are in agreement with Chambers, Redfern & Roberts (1967).

Effect of antidepressant drugs on hypothermic response to noradnamine

The hypothermic response to noradnamine provided a quantitative effect which could be used for the determination of the interactions between antidepressant and

other compounds and noradnamine. Figure 2 shows the effect on the noradnamine hypothermia of pretreatment with imipramine (10 mg/kg) and amitriptyline (10 mg/kg) which antagonized, and nortriptyline (10 mg/kg) and desmethylimipramine (10 mg/kg) which reversed, the hypothermia produced by noradnamine (20 μg) injected into the lateral ventricles of mice.

The effect of nortriptyline (1, 5 and 10 mg/kg) given intraperitoneally 2 hr after noradnamine (20 μg) injected into the lateral ventricles is shown in Fig. 3. The

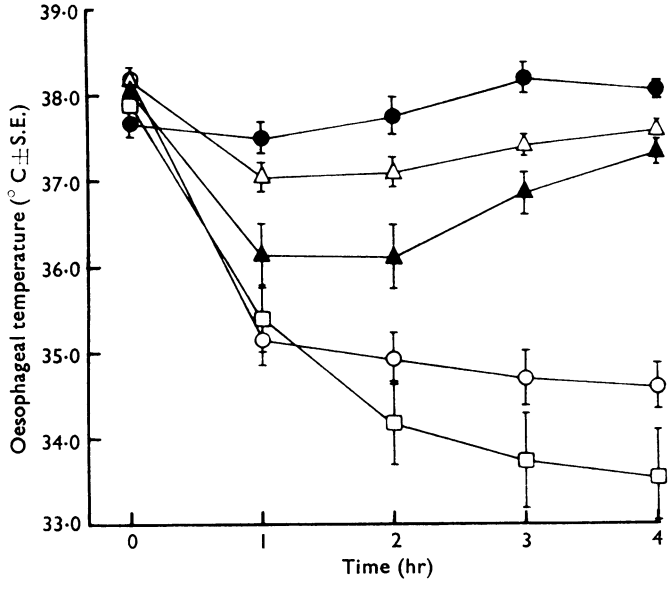

FIG. 1. Record of the fall in oesophageal temperature in conscious mice after noradnamine (5, 10, 20 and 40 μg) injected intraventricularly. Room temperature 19° C. ●——●, Saline; △——△, noradnamine 5 μg; ▲——▲, noradnamine 10 μg; ○——○, noradnamine 20 μg; □——□, noradnamine 40 μg. $n=10$ in each case.

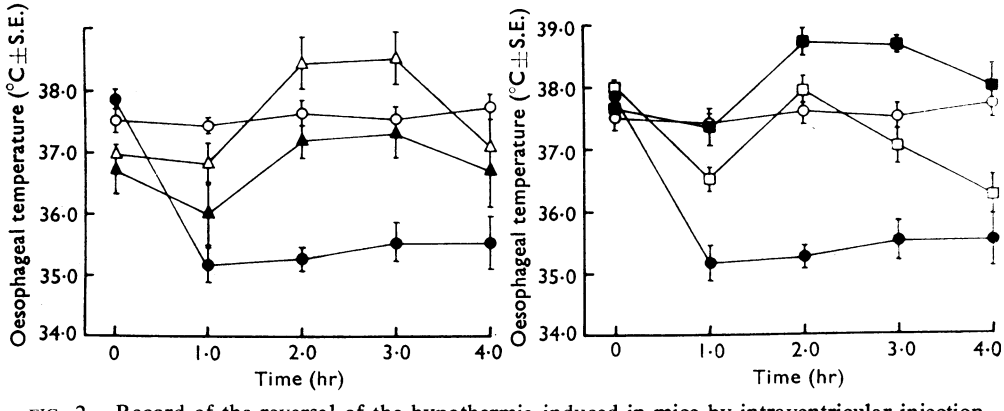

FIG. 2. Record of the reversal of the hypothermia induced in mice by intraventricular injection of noradnamine (20 μg) by: ▲——▲, amitriptyline (10 mg/kg); □——□, imipramine (10 mg/kg); ■——■, desmethylimipramine (10 mg/kg); △——△, nortriptyline (10 mg/kg), given orally 1 hr before the noradnamine injection. ○——○, Saline 1 hr; saline lat. vent.; ●——●, saline 1 hr; noradnamine 20 μg lat. vent. Room temperature 19° C. $n=10$ in each case.

established hypothermia was antagonized by nortriptyline (1 and 5 mg/kg) given intraperitoneally and reversed to a hyperthermia by nortriptyline (10 mg/kg).

Effect of nortriptyline on hypothermic log-dose response curve to noradnamine

Having established that antidepressant drugs antagonized the hypothermic action of noradnamine, investigations were then directed towards finding evidence for a competitive action between noradnamine and the imipramine-like antidepressant drugs. Figure 4 shows the effect on the hypothermic response to noradnamine of nortriptyline (5, 10 and 20 mg/kg) given orally 1 hr before the noradnamine (5, 10, 15, 20 and 25 μg) injected directly into the lateral ventricles. Nortriptyline (5, 10 and 20 mg/kg) caused similar parallel shifts of the log dose-response curve for noradnamine to the right, which could be considered as being compatible with a competitive antagonism between noradnamine and nortriptyline.

Dissociation of behavioural and hypothermic actions of noradnamine

The first indication that the imipramine-like antidepressant drugs did not antagonize all the effects of noradnamine was suggested by the differing action of antidepressant drugs on the hypothermia and on the convulsive behavioural symptoms of noradnamine. In contrast to their effect on the hypothermia, pretreatment with imipramine (10 mg/kg), amitryptyline (10 mg/kg), desmethylimipramine (10 mg/kg) and nortriptyline (10 mg/kg) given intraperitoneally either 60 or 15 min before the injection of noradnamine (20 μg) into the lateral ventricles did not antagonize the convulsive behavioural syndrome induced by noradnamine, whereas these amounts abolished the hypothermic effect of noradnamine.

Chlordiazepoxide (10 mg/kg), pargyline (10 mg/kg), promethazine (10 mg/kg), mepyramine (10 mg/kg), atropine (10 mg/kg) or chlorpromazine (5 mg/kg) given intraperitoneally failed to modify either the hypothermia or the behavioural effects of noradnamine. Phenobarbitone sodium (40 mg/kg) given intraperitoneally 15 min before the intraventricular injection of noradnamine (20 μg) abolished the convulsive syndrome but did not modify the hypothermia.

Effect of route of administration of nortriptyline on reversal of noradnamine hypothermia

Neither the convulsive activity nor the hypothermia induced by injection of noradnamine into the lateral ventricle were produced by intraperitoneal injections of noradnamine (10 mg/kg), which was approximately 10 times the quantity that was required to produce a profound hypothermia and marked convulsive activity when injected into the lateral ventricle. This indicated that the hypothermia is centrally mediated and suggests that the antidepressant reversal of it is also of central origin, if the antagonism of the hypothermia is competitive in nature. However, Figure 5 shows that the minimal effective dose of nortriptyline required to produce a significant reversal of the hypothermic effect due to noradnamine (20 μg) injected into the lateral ventricles was 1·0 mg/kg, irrespective of whether the nortriptyline was injected subcutaneously or directly into the ventricles. Therefore, in contrast to the hypothermic response to noradnamine, these results indicate that the reversal of the hypothermia by nortriptyline is mediated peripherally.

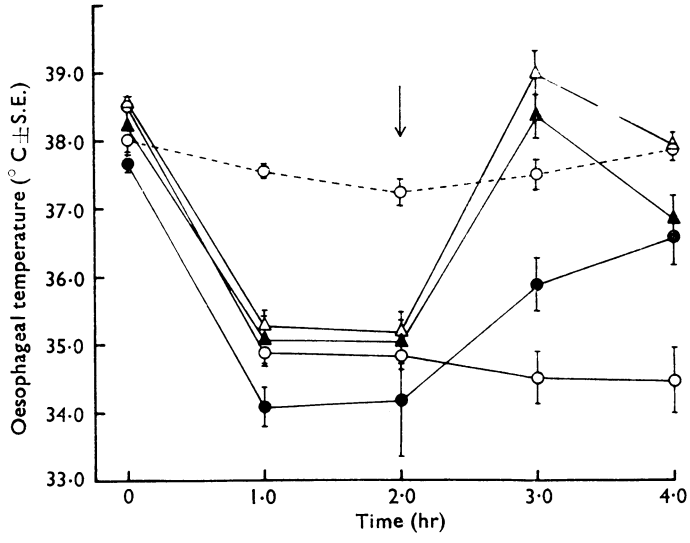

FIG. 3. Record of the reversal of the hypothermia induced by intraventricular injection of noradnamine (20 μg) by nortriptyline (1, 5 and 10 mg/kg) given intraperitoneally 2 hr after the injection of noradnamine. Room temperature 19° C. ○——○, Noradnamine 20 μg; saline 2 hr (n=20); ○- - -○, saline; saline 2 hr (n=15); ●——●, nortriptyline 1 mg/kg (n=8); ▲——▲, nortriptyline 5 mg/kg (n=10); △——△, nortriptyline 10 mg/kg (n=20).

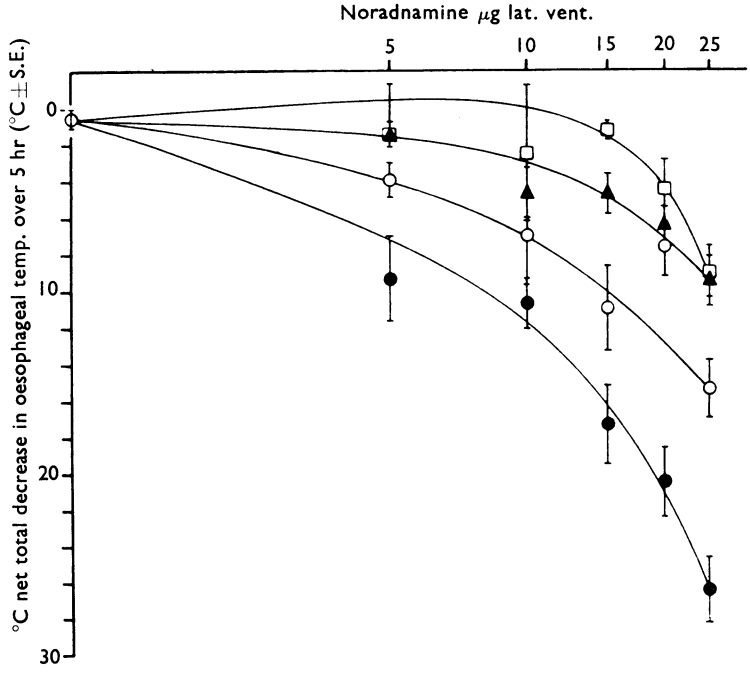

FIG. 4. Record of antagonism of hypothermic effect of noradnamine (5, 10, 15, 20 and 25 μg) injected intraventricularly by nortriptyline (5, 10 and 20 mg/kg) orally given 1 hr before the injection of noradnamine. Room temperature 19° C. ●——●, Saline (n=20–30 per point); ○——○, nortriptyline 5 mg/kg (n=10–15 per point); ▲——▲, nortriptyline 10 mg/kg (n=20–30 per point); □——□, nortriptyline 20 mg/kg (n=10–20 per point).

Comparison of effectiveness of drugs against noradnamine and reserpine hypothermia

A consequence of the Roberts & Broadley (1965) hypothesis was that the hypothermic effect of reserpine was mediated by the accumulation of noradnamine. Hence all drugs which antagonized noradnamine hypothermia should cause a similar antagonism of reserpine hypothermia. Table 1 shows that although the majority

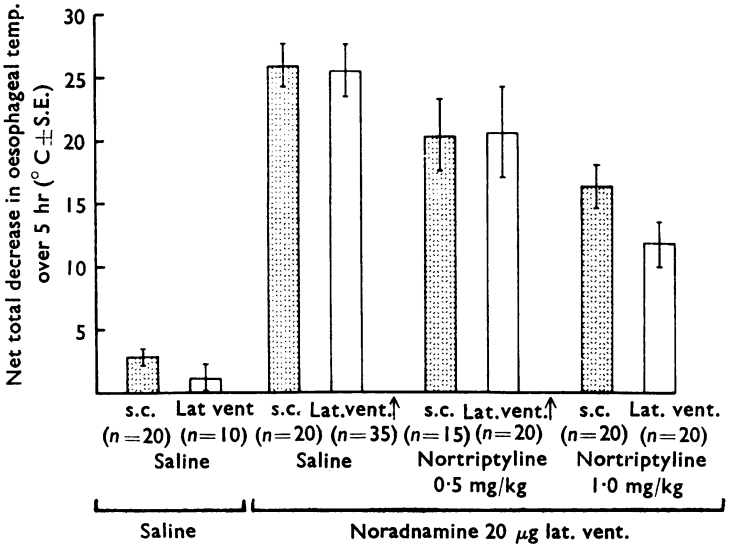

FIG. 5. Histogram of the effect of pretreatment with nortriptyline (1·0 and 0·5 mg/kg) given either subcutaneously or directly into the lateral ventricle 30 min before on the net total decrease in oesophageal temperature (° C) over 5 hr induced by an injection of noradnamine (20 μg) into the lateral ventricle in mice. Room temperature 19° C.

TABLE 1. *Degree of antagonism of the hypothermia induced with either reserpine or noradnanime by a variety of pharmacological agents*

Drug	Dose (mg/kg i.p.)	Degree of antagonism: Noradnamine hypothermia	Degree of antagonism: Reserpine hypothermia
Amitriptyline	10	+++	+++
Imipramine	10	+++	+++
Desmethylimipramine	10	+++	+++
Nortriptyline	10	+++	+++
Amphetamine	10	+++	+++
Methylphenidate	10	+++	+++
Cocaine	10	+/++	++
Nialamide	10	+	0
Tranylcypromine	10	+++	+++
Pargyline	10	0	0
Tripelennamine	10	+/++	++/+++
Phenindamine	10	+++	+++
Diphenhydramine	10	+	+
Chlordiazepoxide	10	0	0
Chlorpromazine	5	0	+/++

Scoring system:
Noradnamine hypothermia
+++ >70% return to control body temperature after 60 min. ++ 50–70% return to control body temperature after 60 min. + 25–50% return to control body temperature after 60 min. 0 <25% return to control body temperature after 60 min.
Reserpine hypothermia
Sum of net mean increases in body temperature (° C) measured hourly for 5 hr.
+++ >20° C. ++ 10–20° C. + 6–10° C. 0 0–5° C.
Drugs administered 2 hr after noradnamine (20 μg) intraventricularly.

of drugs antagonized both noradnamine and reserpine hypothermia, chlorpromazine (5 mg/kg) given intraperitoneally antagonized reserpine hypothermia, but potentiated the hypothermic effect of noradnamine.

Effect of noradnamine on brain noradrenaline and 5-hydroxytryptamine levels

Falls in brain amine levels are often associated with the hypothermic response produced by drugs, so the effect of noradnamine on brain amine levels was investigated. Figure 6 illustrates the effect of an injection of noradnamine (20 μg) into the lateral ventricle in groups of ten pairs of mice on brain noradrenaline levels and oesophageal temperature 1, 2, 3, 4 and 5 hr after the injection. After 2 hr the brain noradrenaline level was 74% of normal and the oesophageal temperature had fallen by 3·5° C. These reductions were maintained over the following 3 hr. The injection

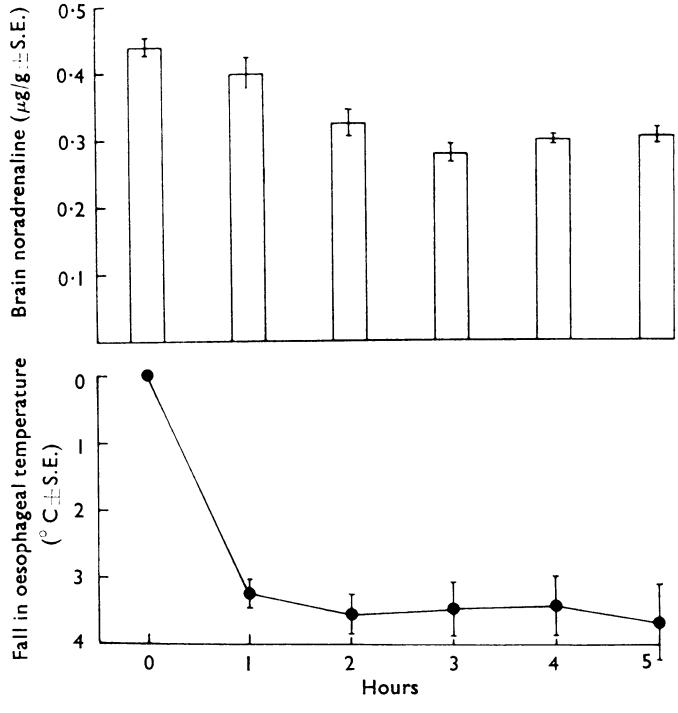

FIG. 6. Record of the effect of an injection of noradnamine (20 μg) into the lateral ventricle in mice on brain noradrenaline levels and oesophageal temperature 1, 2, 3, 4 and 5 hr after the injection of noradnamine. Room temperature 19° C.

TABLE 2. *Effects of noradnamine on oesophageal temperature and heart and brain noradrenaline levels in mice*

Treatment	Route	No. paired mice	Time (hr)	Initial temp. (°C ± S.E.)	Final temp. (°C ± S.E.)	Brain noradrenaline (μg/g ± S.E.)	Heart noradrenaline (μg/g ± S.E.)
Saline	i.p.	5	2	37·37 ± 0·25	35·85 ± 0·14	4·11 ± 0·31	3·55 ± 0·53
Noradnamine 10 mg/kg	i.p.	5	2	36·85 ± 0·20	36·50 ± 0·29	4·30 ± 0·22	3·50 ± 0·44
Saline	Lat. vent.	5	2	38·07 ± 0·25	36·47 ± 0·23	4·17 ± 0·18	3·65 ± 0·44
Noradnamine 20 μg	Lat. vent.	5	2	36·72 ± 0·25	32·90 ± 0·47	3·47 ± 0·09 (P=0·02–0·01)	4·27 ± 0·31 (P=0·4–0·3)

or noradnamine (20 μg) directly into the lateral ventricle had no effect on noradrenaline levels in the heart (Table 2). This association between the depression of body temperature and brain noradrenaline suggests the possibility that the hypothermic effect of noradnamine may be caused by an increase in free noradrenaline levels within the brain. Intraperitoneal injections of noradnamine (10 mg/kg) had no effect on noradrenaline levels in the heart or the brain (Table 2).

Phenobarbitone sodium (40 mg/kg) given subcutaneously 30 min before noradnamine (20 μg) was injected into the lateral ventricle, abolished the convulsive activity of noradnamine, but had no effect on the brain depletion of noradrenaline (Table 3). This, taken together with the fact that falls in brain noradrenaline levels are recorded before convulsive activity is apparent, indicates that the falls in brain noradrenaline levels are not a consequence of the convulsive activity of noradnamine.

In contrast to the depletion of brain noradrenaline levels, a 160% increase in brain levels of 5-hydroxytryptamine was recorded 2 hr after noradnamine (20 μg) injected directly into the lateral ventricles (Table 4).

TABLE 3. *Absence of an effect of anticonvulsive amounts of phenobarbitone sodium on the depletion of brain noradrenaline by noradnamine in mice*

Treatment	Dose (mg/kg s.c. 30 min)	Lateral ventricle	No. Mouse Brains (paired)	Time (hr)	Brain noradrenaline (μg/g ± S.E.)	% Depletion
Saline	—	Saline	5	2	3·75 ± 0·15	—
Phenobarbitone sodium	40	Saline	5	2	3·98 ± 21	—
Saline	—	Noradnamine 20 μg	5	2	2·71 ± 0·20	28
Phenobarbitone sodium	40	Noradnamine 20 μg	5	2	3·06 ± 0·15	23

TABLE 4. *Elevation of brain 5-hydroxytryptamine content by noradnamine given intraventricularly in mice*

Treatment	Route	No. mice	Time (hr)	Brain 5-hydroxytryptamine (μg/g ± S.E.)
Saline	Lateral ventricle	15	2	0·569 ± 0·061
Noradnamine 20 μg	Lateral ventricle	17	2	1·488 ± 0·32

TABLE 5. *Absence of an effect of nortriptyline on the decrease in brain noradrenaline content induced by noradnamine in mice*

Treatment	Dose (mg/kg p.o. 1 hr)	Lateral ventricle	No. Mouse brains (paired)	Time (hr)	Brain noradrenaline (μg/g ± S.E.)	% Fall in NA levels
Saline	—	Saline	10	2	4·36 ± 0·11	—
Saline	—	Noradnamine 20 μg	9	2	2·95 ± 0·11	30
Nortriptyline	10	Noradnamine 20 μg	10	2	2·85 ± 0·11	34

Effect of nortriptyline and brain depletion of noradrenaline by noradnamine

If the depletion of brain noradrenaline was responsible for the hypothermic response after the injection of noradnamine, it was possible that antidepressant drugs modified the effect. To test this possibility, nortriptyline (10 mg/kg) was given orally 1 hr before injecting noradnamine (20 μg) into the lateral ventricle, and it had had no effect on the depletion of brain noradrenaline by noradnamine after 2 hr

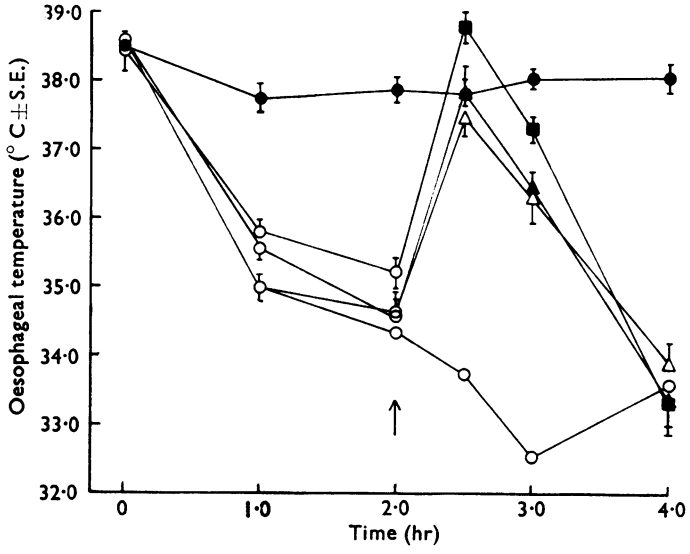

FIG. 7. Record of the reversal of the hypothermia induced by intraventricular injection of noradnamine (20 μg) in mice by noradrenaline (1, 2 and 4 mg/kg) given subcutaneously 2 hr after the injection of noradnamine. Room temperature 19° C. ●——●, Saline lat. vent.; Saline 2 hr (n=15); ○——○, noradnamine 20 μg lat. vent.; saline 2 hr (n=25); △——△, noradrenaline 1 mg/kg (n=25); ▲, noradrenaline 2 mg/kg (n=25); ■——■, noradrenaline 4 mg/kg (n=25).

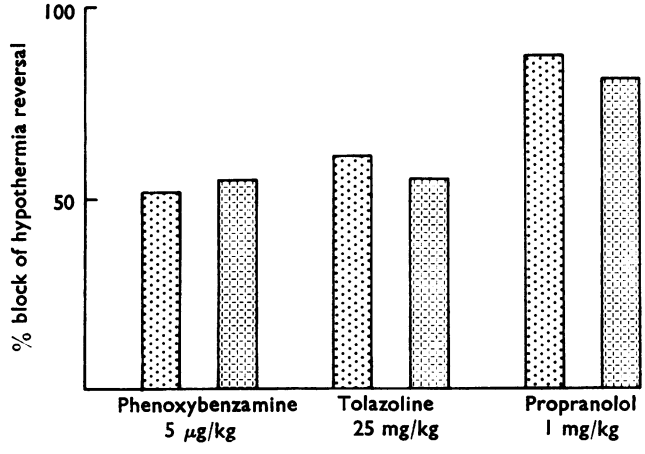

FIG. 8. Histogram of the average percentage reduction by phenoxybenzamine (5 mg/kg), tolazoline (25 mg/kg) and propranolol (1 mg/kg) of the reversal of the hypothermia induced by intraventricular injection of noradnamine (20 μg) given 2 hr previously, by nortriptyline (10 mg/kg) given intraperitoneally (▨) and noradrenaline (2 mg/kg) given subcutaneously (▩). The adrenoceptive blocking drugs were administered simultaneously with the nortriptyline and 45 min before the noradrenaline.

(Table 5). Since this amount of nortriptyline abolished the hypothermic effect of noradnamine, the reversal of the hypothermia by nortriptyline may not be attributed to an effect of nortriptyline on the depletion of brain noradrenaline by noradnamine.

Effect of noradrenaline given subcutaneously on noradnamine hypothermia

The depletion of brain noradrenaline levels by noradnamine may lead to increased peripherally circulating levels of noradrenaline. The possibility that antidepressant drugs could potentiate the hyperthermic effect of this circulating noradrenaline, thus overcoming the hypothermia in a similar manner to the reversal of the hypothermic response to centrally injected noradrenaline by antidepressant drugs (Cowell & Davey, 1968) has been considered.

Noradrenaline (1, 2 and 4 mg/kg) given subcutaneously 2 hr after injection of noradnamine (20 μg) into the lateral ventricle reversed the established hypothermic response (Figure 7). This reversal was similar to that seen after treatment with antidepressant drugs.

Effect of α- and β-adrenoceptive receptor blocking agents on the reversal of noradnamine hypothermia by nortriptyline and noradrenaline given subcutaneously

Phenoxybenzamine (5 mg/kg), tolazoline (25 mg/kg) and propranolol (1 mg/kg) given intraperitoneally simultaneously with nortriptyline (10 mg/kg) given intraperitoneally, and 45 min prior to noradrenaline (2 mg/kg) given subcutaneously, partially blocked the reversal by nortriptyline and noradrenaline of the hypothermia induced by noradnamine (20 μg) given 2 hr previously into the lateral ventricle (Figure 8). This antagonism strengthens the hypothesis that antidepressant drugs antagonize the hypothermic effect of noradnamine by potentiating the peripheral hyperthermic effect of noradrenaline.

Discussion

Roberts & Broadley (1965) proposed that endogenous depression was the consequence of malfunction of the enzyme catechol-O-methyl transferase, which led to the accumulation of noradnamine, a metabolite of noradrenaline. It was suggested that the imipramine-like antidepressant drugs were competitive antagonists of noradnamine. Investigations were therefore directed towards finding such an antagonism.

The symptomatology in mice injected with noradnamine showed little evidence of depressive activity, the predominant feature being the marked convulsant effects. In mice a profound hypothermic response was recorded, which was used to measure the effect of the antidepressant drugs on the noradnamine response. Increasing doses of nortriptyline produced a parallel shift of the hypothermic log dose-response curve for noradnamine to the right. Although this is compatible with a competitive antagonism, the fact that antidepressant drugs antagonized the hypothermia, but had no effect on the convulsive activity, threw the first doubts on the theory of a direct competitive antagonism between noradnamine and imipramine-like compounds.

Since no hypothermic or convulsive effects are obtained after peripheral administration of noradnamine, the hypothermia was considered to be a central effect. In contrast, the results suggest that the nortriptyline reversal of the hypothermia was mediated peripherally, because the injection of nortriptyline directly into the lateral ventricle did not increase the effectiveness of this drug in antagonizing the hypothermic response to noradnamine. This is clearly incompatible with a competitive mechanism of action between antidepressant drugs and noradnamine.

Again, contrary to the Roberts & Broadley (1965) hypothesis, chlorpromazine was found to antagonize reserpine hypothermia, but to potentiate noradnamine hypothermia. It therefore seems clear that noradnamine formation does not play a rôle in the hypothermic response to reserpine.

The falls in body temperature after noradnamine treatment could result from increased central levels of circulating noradrenaline, since a correlation has been demonstrated between falls in brain noradrenaline levels and falls in body temperature. Pretreatment with nortriptyline failed to modify this depletion, so the reversal of the hypothermia by antidepressant drugs may not be explained in terms of an effect on the brain depletion of noradrenaline by noradnamine.

Since a competitive mechanism of action for antidepressant drugs against noradnamine hypothermia had been ruled out, investigations were directed towards revealing a possible peripheral mode of action. The fact that brain depletion of noradrenaline could result in increased peripherally circulating levels of noradrenaline suggested that antidepressant drugs may potentiate the hyperthermic effects of this peripherally circulating noradrenaline, thus overcoming the hypothermia, in a similar manner to the reversal of the hypothermic response to centrally injected noradrenaline by antidepressant drugs or peripherally injected noradrenaline (Cowell & Davey, 1968). Noradrenaline given subcutaneously was subsequently demonstrated to antagonize noradnamine hypothermia in a similar manner to antidepressant drugs. In addition, both the reversal by noradrenaline and that by nortriptyline were blocked by α- and β-adrenoceptive receptor blocking agents.

It is therefore suggested that imipramine-like antidepressant drugs antagonize noradnamine hypothermia by potentiating the hyperthermic effects of increased circulating levels of noradrenaline, resulting from the depletion of noradrenaline stores by noradnamine. No evidence for a direct central antagonism between antidepressant drugs and noradnamine has been found. Further evidence against a competitive action for the antidepressant drugs in this antagonism was the finding that the imipramine-like drugs not only antagonized the hypothermia induced by noradnamine, but reversed this to a hyperthermia.

I should like to thank Dr. M. J. Davey for his advice and encouragement, and Miss Doreen Brown, Miss June Ottaway and Mr. M. Sitton for their invaluable technical assistance.

REFERENCES

ASKEW, BERYL M. (1963). A simple screening procedure for imipramine-like antidepressant agents. *Life Sci., Oxford*, **10**, 725–730.

BRITTAIN, R. T. & SPENCER, P. S. J. (1964). Measurement of body temperature in conscious small animals by means of oesophageal thermocouple. *J. Pharm. Pharmac.*, **16**, 497–499.

CHAMBERS, D. M., REDFERN, P. H. & ROBERTS, D. J. (1967) Some observations on the central pharmacological actions of noradnamine, a proposed depressive catabolite of noradrenaline. *Int. J. Neuropharmac.*, **6**, 417–422.

COWELL, PAULINE & DAVEY, M. J. (1968). The reversal of the central effects of noradrenaline by antidepressant drugs in mice. *Br. J. Pharmac.*, **34**, 159–168.

HALEY, T. J. & MCCORMICK, W. G. (1957). Intracerebral injection in the conscious mouse. *Br. J. Pharmac. Chemother.*, **12**, 12–15.

KAWAZU, M. (1958). Decomposition of adrenaline and its analogues by some mineral acids. No. 4: Decomposition of adrenaline, N-methyladrenaline and 1-(monoalcoxyphenyl)-2-alkylamino ethanols by mineral acids. *Pharm. Soc. Japan*, **78**, 974–977.

KUNTZMAN, R., SHORE, P. A., BOGDANSKI, D. & BRODIE, B. B. (1961). Microanalytical procedures for fluorimetric assay of brain dopa-5HTP decarboxylase, norepinephrine and serotonin, and a detailed mapping of decarboxylase activity in brain. *J. Neurochem.*, **6**, 226–232.

MERRILLS, R. J. (1963). A semi-automatic method for determination of catecholamines. *Analyt. Biochem.*, **6**, 272–282.

ROBERTS D. J. & BROADLEY, K. J. (1965). Treatment of depression. *Lancet*, **1**, 1219–1220.

(Received December 19, 1968)