Role of 5-hydroxytryptaminergic and adrenergic mechanism in antagonism of reserpine-induced hypothermia in mice

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Antagonism of reserpine-induced hypothermia, a standard test for antidepressant agents (Askew 1963; van Riezen & Delver 1971), could reflect inhibition of reuptake of either 5-hydroxytryptamine (5-HT) or noradrenaline since both amines influence temperature regulation. Most tricyclic antidepressants inhibit reuptake of both amines but selective agents have become available. Fluoxetine is a specific inhibitor of 5-HT uptake and its chemical congener, nisoxetine has a greater effect on noradrenaline uptake (Fuller et al 1974). Desipramine (desmethylimipramine) inhibits noradrenaline uptake and clomipramine (chlorimipramine), 5-HT uptake. These pairs of chemically related drugs enabled us to examine directly the role of biogenic amine uptake in the antagonism of reserpine-induced hypothermia in mice.

Male mice (Lai: Cox (Standard) BR), 20–22 g in groups of 5 were kept in hanging wire cages and removed only for injections or measurement of rectal temperature electronically. The mice received reserpine, 4 mg kg^{-1}, i.p., either 16 h before or 30 min after intraperitoneal doses of the uptake inhibitors. Rectal temperature was measured at the times indicated in the Figure and Tables. Mice receiving reserpine at −16 h were dosed at 4 p.m. and their temperatures were read at about 7.30 and 8.00 a.m. the following morning. Since the two readings were similar only the 8 a.m. reading was used in Fig. 1. Ambient temperature varied between 21·4 and 22·8° C. Each experiment included all treatments run in random order on the same day. Nisoxetine, fluoxetine and reserpine (Sandril) were from Eli Lilly and Company; desipramine from Merrell-National Laboratories and clomipramine from Ciba-Geigy. The Duncan multiple range test was used to determine statistical significance of differences among the groups.

Mice treated with reserpine (4 mg kg^{-1}, i.p.) at 4 p.m. were profoundly hypothermic at 7.30 and 8.00 a.m. the next morning. 20 mice received saline as control and initially 10 mice were used for each dose. Temperatures were between 22–28·75° C (n = 132), mean fall, 13·8° C. During the next 4 h the temperature of saline-treated controls increased slowly. One and 2 h after treatment with saline, fluoxetine (3, 10, or 30 mg kg^{-1}, i.p.) or clomipramine (1, 3 or 10 mg kg^{-1}, i.p.) mice had similar levels of hypothermia (Fig. 1). In contrast, the mice that had received the noradrenaline-uptake inhibitors, desipramine or nisoxetine, had substantially higher temperatures. A Duncan multiple range test indicated that temperatures 1 h after injection were significantly higher than controls for mice treated with 3 or 10 mg kg^{-1}, i.p. of either nisoxetine or desipramine ($P < 0.05$). After 2 h and 4 h, all three doses (1, 3 and 10 mg kg^{-1}) of both noradrenaline-uptake inhibitors had effects different from controls ($P < 0.05$). The most striking difference occurred after 2 h. By the 4th hour, probably as a consequence of falling concentrations of drug in brain and plasma, antagonism of the hypothermia was reduced. Metabolism of clomipramine leads to a monomethyl compound that is a noradrenaline-uptake inhibitor whereas desmethyl-fluoxetine specifically inhibits 5-HT (Fuller et al 1978). After 4 h each group of clomipramine-treated mice had temperatures higher than the saline or fluoxetine-treated animals. Although Duncan's multiple range test indicated that

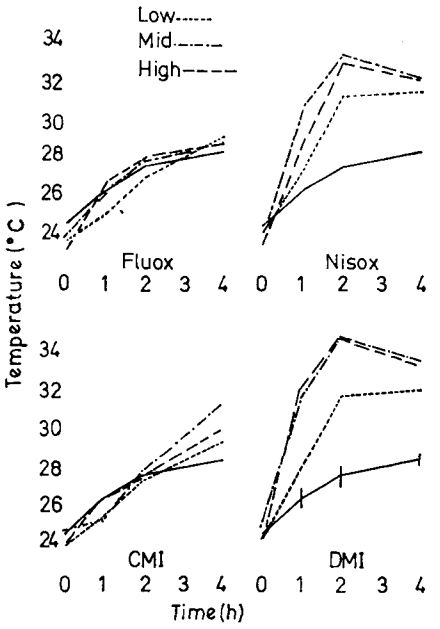

FIG. 1. Effect of uptake inhibitors on hypothermia induced by reserpine 4 mg kg^{-1}, i.p., given at 4 p.m. The uptake inhibitors were injected i.p. at about 8 a.m. the next morning. No dose of either clomipramine (CMI) or fluoxetine (Fluox) resulted in a significant ($P < 0.05$) difference from control (Duncan multiple range test). All temperatures except the 1 h, 1 mg kg^{-1} dose after nisoxetine (Nisox) and desipramine (DMI) treatment were significantly different from control. Doses of desipramine, nisoxetine and clomipramine were 1, 3 and 10 mg kg^{-1}; of fluoxetine, 3, 10 and 30 mg kg^{-1}.

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the separate dose-level groups are not different from the control, Student's *t*-test combining all clomipramine-treated mice demonstrated a significant difference from control.

An analysis of these same results based on changes in temperature of individual mice shows that saline-treated mice increased 4·0 ± 0·51° C after 4 h. Clomipramine-treated mice had increases of 5·5 ± 0·68, 7·4 ±0·67 and 6·0 ± 0·89° C for the 1, 3 and 10 mg kg^{-1} doses. The latter two were significant ($P < 0·01$) by Student's *t*-test. For the fluoxetine mice, only an increase of 5·6 ± 0·76° C for the 30 mg kg^{-1} group was significant ($P < 0·05$). When computed on the basis of change in temperature all the changes, including the first hour reading, were significant for nisoxetine and desipramine ($P < 0·01$ in all six dose groups).

When mice received resperine, 4 mg kg^{-1}, i.p., in the morning, their rectal temperature fell progressively. Pretreatment with nisoxetine or desipramine antagonized hypothermia at 90 min (Table 1). In contrast, temperature fell in mice pretreated with the 5-HT uptake inhibitors, fluoxetine or clomipramine. After 3·5 h, temperatures of the saline controls were lower than all groups except the fluoxetine.

Table 1. Temperature of mice treated with drug and followed 30 min later with reserpine

Reserpine 0	90 min	210 min
Saline		
39·0 ± 0·20[a]	36·5 ± 0·30[b]	30·4 ± 0·64[b]
Clomipramine 10 mg kg^{-1}		
38·6 ± 0·13[a,b]	36·5 ± 0·22[b]	32·5 ± 0·39[a]
Fluoxetine 30 mg kg^{-1}		
35·9 ± 0·38[c]	33·8 ± 0·52[c]	28·9 ± 0·45[c]
Nisoxetine 10 mg kg^{-1}		
38·1 ± 0·16[b]	37·7 ± 0·14[a]	33·6 ± 0·37[a]
Desipramine 10 mg kg^{-1}		
38·4 ± 0·27[a,b]	38·1 ± 0·19[a]	35·1 ± 0·23[d]

Ten mice per group—reserpine 4 mg kg^{-1}, i.p., injected 30 min after uptake inhibitor.
Duncan multiple range test—Values with the same superscript letter are not different from each other at the time indicated ($P < 0·05$).

In this experiment we used only a single dose of each uptake inhibitor. The large (30 mg kg^{-1}, i.p.) dose of fluoxetine itself caused hypothermia. Another experiment showed that fluoxetine 3 and 10 mg kg^{-1}, i.p., like saline, did not affect rectal temperature alone (no significant differences from control values) and also did not antagonize reserpine-induced hypothermia (no significant changes from reserpine values).

Hypothermia, like other reserpine-induced effects, is a consequence of biogenic amine depletion. Agents that increase availability of catecholamine mediators and sympathomimetic amines antagonize both the sedation and the hypothermia (van Riezen & Delver 1971). Carlsson et al (1957) found that dopa reversed reserpine-induced sedation and ptosis, that 5-HTP did not, but that a combination of the two amino acids was more effective than dopa alone. Dopa also antagonizes the hypothermia (Jori & Garattini 1965). Monoamine oxidase inhibitors, which increase concentrations of biogenic amines, block the development of reserpine-induced hypothermia. But these drugs cause overt stimulation and may increase temperature when given alone in sufficient dose. Antidepressant agents antagonize reserpine hypothermia, cause little, if any, increase in spontaneous activity and may lower body temperature when given alone.

Garattini & Jori (1967) reviewed the interaction between tricyclic antidepressants and reserpine on body temperature and concluded that the effect is 'mediated through the central sympathetic system'. Peripheral effects of noradrenaline such as increase in blood pressure (Osborne & Sigg 1960) and hyperthermia (Jori & Garattini 1965) are enhanced by pretreatment with imipramine. Adrenoceptor blocking agents (Jori et al 1966a) and inhibitors of noradrenaline synthesis (Jori et al 1966b) interfere with the reversal by desipramine of reserpine hypothermia. In general, the secondary amines, desipramine and nortriptyline are more effective antagonists of the hypothermia than the corresponding tertiary amines imipramine and amitriptyline (Askew, 1963; Garattini et al 1962). Ross & Renyi (1975) reported that simultaneous uptake of noradrenaline and 5-HT by synaptosomes was selectively inhibited by antidepressants. Secondary amines were more effective inhibitors of noradrenaline uptake and the tertiary amines, of 5-HT uptake.

Our results using more selective inhibitors of amine uptake provide further support for the role of adrenergic mechanisms in the reversal of reserpine hypothermia in mice. Fluoxetine, a specific 5-HT inhibitor whose major metabolite is also 5-HT-specific, did not prevent reserpine hypothermia when injected before reserpine. It had scarcely any effect on the recovery of body temperature when injected during profound post-reserpine hypothermia. Clomipramine, which is metabolized to an agent that inhibits noradrenaline uptake, did affect recovery after a latent period. Both nisoxetine and desipramine were effective in both assays. Thus, although the intracerebral injection of 5-HT increases body temperature and similar injection of noradrenaline reduces body temperature, it seems clear that antagonism of reserpine hypothermia by antidepressant drugs in mice depends on adrenergic mechanisms.

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Effect of lysolecithin on the systemic arterial blood pressure of anaesthetized rats

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Lysolecithin (LPC), a naturally occurring glycerophosphatide found in various tissues and fluids, has strong haemolytic activity. It is formed by hydrolysis of lecithin by phospholipase A_2 with liberation of fatty acids.

Middleton & Phillips (1963) reported that LPC causes non-competitive inhibition of the effects of various stimuli on smooth muscle. From this finding LPC might be expected to influence the cardiovascular system of animals. Khairallah & Page (1960) reported that a preparation obtained from dog incubated plasma had a pressor effect on anaesthetized rats and suggested that the effect was due to LPC; conversely they found that synthetic stearoyl-LPC elicited a depressor response in the rats. Since the vasoactivity of LPC is uncertain, we have examined the vaso-activities of LPC with various fatty acid moieties on the systemic arterial blood pressure of anaesthetized rats.

Male Wistar strain rats, 240–260 g, were anaesthetized with sodium pentobarbitone (40 mg, kg^{-1}, i.p.). The arterial blood pressure was recorded through a cannula in the left carotid artery with a mercury manometer or a pressure transducer (Nihon Kohden MPU-0·5) coupled to a multipurpose polygraph (Nihon Kohden RM-45). The heart rate was recorded with a cardiotachometer triggered by the arterial pressure wave. Test substances in 0·15 ml of physiological saline (0·9% w/v, NaCl solution) were injected into the left femoral vein through a cannula and the cannula was then flushed with 0·1 ml of saline. When necessary, emulsions of substances were prepared by sonication (e.g. stearoyl-LPC).

Lauroyl-, myristoyl- and palmitoyl-LPC(1-acyl-*sn*-glycero-3-phosphocholine) and didecanoyl- and distearoyl-lecithin(1,2-di-acyl-*sn*-glycero-3-phosphocholine) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Oleoyl-, linoleoyl- and linolenoyl-LPC were supplied from Sardary Research Laboratories (London, Ontario, Canada). Didecanoyl- and distearoyl-lecithin were hydrolysed with phospholipase A_2 (Bee Venom, Sigma) to the respective LPCs as described by Shipolini et al (1971). Before use, the purity of each LPC was confirmed by t.l.c. and fatty acid analysis was carried out by gas chromatography on samples prepared by methanolysis. Values are given as means (± s.e.m.) of results in at least five experiments unless otherwise stated.

On intravenous administration all the LPCs tested decreased both the systolic and diastolic arterial blood pressure, and none of the compounds had the pressor activity suggested by Khairallah & Page (1960).

The log dose-depressor response relation of saturated LPCs are shown in Fig. 1.

Both the depressor responses and their durations

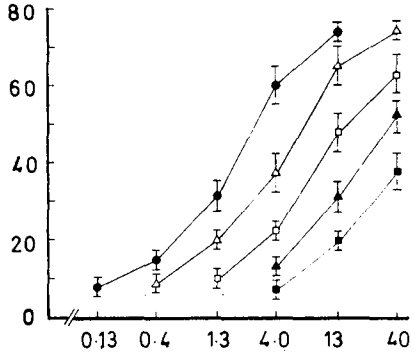

FIG. 1. Dose-response relation of saturated LPCs. The fatty acid composition of the LPCs is indicated by: ■ $C_{10:0}$, □ $C_{12:0}$, ● $C_{14:0}$, △ $C_{16:0}$, ▲ $C_{18:0}$. Results are expressed as means ± s.e.m. Ordinate: Decrease in carotid arterial blood pressure (mm Hg). Abscissa: Intravenous lysophosphatidyl choline (μmol kg^{-1}).

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