Antagonism of apomorphine and lergotrile hypothermia

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There is now considerable evidence to suggest that more than one type of dopamine receptor exists within the central nervous system (Iversen, 1978, Tye, *et al.*, 1977). It has also been reported that apomorphine and the ergot dopamine agonists may act as selective agonists for the different dopamine receptors. Since dopamine agonists have been shown to lower body temperature (Fuxe & Sjoqvist 1972, Cox & Lomax 1977, Cox & Lee 1977) we have examined the hypothesis that different dopamine antagonists may preferentially antagonise the hypothermic response produced by either apomorphine or lergotrile.

Male albino MFI mice (20–30 g) were used in all the experiments. Rectal temperature was measured immediately prior to and every 30 min for 90 min following treatment with apomorphine, lergotrile or saline. In some experiments animals were pretreated with haloperidol, pimozide or the respective vehicle 1 h prior to the start of the experiment.

Apomorphine (5 mg/kg) and lergotrile (5 mg/kg) produced a fall in body temperature of 3.5 to 5°C. Haloperidol (0.4 mg/kg and 1 mg/kg) antagonised the hypothermic response produced by apomorphine (5 mg/kg, $P < 0.01$). Haloperidol (0.5 mg/kg) partially antagonised the hypothermic response produced by lergotrile (5 mg/kg, $P < 0.01$), however higher doses (1 mg/kg and 2 mg/kg s.c.) had no effect. Pimozide (0.5 mg/kg and 1 mg/kg s.c.) antagonised the hypothermic response produced both by apomorphine (5 mg/kg) and lergotrile (5 mg/kg, $P < 0.01$). A higher dose of pimozide (2 mg/kg) partially antagonised the fall in body temperature produced by lergotrile (5 mg/kg s.c., $P < 0.05$), but had no effect on the hypothermic response produced by higher doses. Haloperidol (0.2 mg/kg–1.5 mg/kg s.c.) and pimozide (0.5 and 1 mg/kg) cause a shift to the right of the dose response curve for apomorphine, however a similar effect for lergotrile only occurs when doses of haloperidol (0.5 mg/kg) and pimozide (0.5 mg/kg and 1 mg/kg) are used, higher doses of either neuroleptic having no effect. This work suggests that the hypothermic responses produced by apomorphine and lergotrile are mediated by different mechanisms.

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Specific [^{3}H]-imipramine binding in rat brain

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Tricyclic antidepressants inhibit the neuronal uptake of noradrenaline and serotonin (Glowinski & Axelrod, 1964). In addition they interact directly with muscarinic cholinoceptors (Snyder & Yamamura, 1977), alpha-adrenoceptors (U'Prichard, Greenberg, Sheenan & Snyder, 1978), serotonin (Bennett & Aghajanian, 1975) and histamine (Green & Maayani, 1977) receptors. Which, if any, of these actions are responsible for the primary therapeutic activity of these drugs is unclear. Following the success of receptor binding techniques in opening a new approach to the study of the mechanism of action of benzodiazepines (Squires & Braestrup, 1977; Möhler & Okada, 1977) we decided to investigate the possible existence of specific high affinity binding sites for tricyclic antidepressants. To date studies with [^{3}H]-amitriptyline or [^{3}H]-imipramine have shown only non-specific (Rehavi & Sokolovsky, 1978) or low affinity binding (O'Brien, Spirt & Horst, 1978).

The binding of [^{3}H]-imipramine was measured by incubating washed rat brain membranes at a final

concentration of 30 mg original wet tissue weight/ml with [^{3}H]-imipramine (29.8 Ci/mmole N.E.N. Chemicals) for 60 min at 0°C. After this time 100 µl of the incubation medium was rapidly diluted into 5 ml of ice-cold buffer and filtered through Whatman GF/F glassfibre filters. The filters were washed with 3 × 5 ml ice-cold buffer, dried and the radioactivity counted by liquid scintillation spectrometry. Specific binding was defined as that inhibited by the presence of desipramine (10 µM) and represented 55% of the total binding at [^{3}H]-imipramine (5 nM).

The specific binding of [^{3}H]-imipramine was saturable, giving a linear Scatchard plot. The mean dissociation affinity constant, Kd, calculated from 13 such Scatchard plots, using membranes prepared from cerebral cortex, was 4.04 ± 0.52 nM (mean + s.e. mean). The maximal binding, Bmax, was 13.82 ± 1.29 pmoles/g original wet tissue weight. The binding rapidly reached equilibrium, t being 5 min at 0°C with [^{3}H]-imipramine (2.5 nM). The binding was unevenly distributed in the brain with a maximum difference of nearly 8 fold between the richest region, the hypothalamus, (Kd, 5.21 ± 3.4 nM; Bmax, 15.97 ± 2.71 pmoles/g original wet tissue weight, n = 4), and the poorest, the cerebellum (Kd, 8.0 ± 3.1 nM; Bmax, 2.20 ± 0.39 pmoles/g original wet tissue weight, n = 3). No specific [^{3}H]-imipramine binding could be detected in the heart.

The binding is potently inhibited by tricyclic antidepressants such as amitriptyline (IC_{50}, 25 nM) and protriptyline (IC_{50}, 20 nM), and less strongly by atypical antidepressants such as iprindol (IC_{50}, 5.5 µM) and mianserine (IC_{50}, 20 µM). Some monoamine uptake inhibitors and certain other compounds also weakly inhibit the binding but there does not appear to be any obvious relationship between the inhibition of [^{3}H]-imipramine binding and any of the major pharmacological activities so far investigated.

Thus the specific binding of [^{3}H]-imipramine is of high-affinity, rapid and reversible. It is assymetrically distributed in the brain and absent in the heart. In addition we have observed that chronic treatment with desipramine (10 mg kg^{-1} day^{-1}) for three weeks reduced the binding of [^{3}H]-imipramine in the cortex by about 42% (Control, Kd, 3.95 + 1.15 nM; Bmax, 11.84 + 1.11 pmoles/g original wet tissue weight, n = 6: desipramine-treated, Kd, 3.52 ± 0.46 nM; Bmax, 6.87 ± 0.64 pmoles/g original wet tissue weight, n = 7; difference of Bmax 41.97% $P < 0.005$). It is concluded that this binding of imipramine may be related to the site of action of tricyclic antidepressant drugs and as such could open up a new approach to the study of the biochemical basis of depression and of the mechanism of action of antidepressant drugs.

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Effects of chronic antidepressant administration on the synthesis of monoamines in rat brain

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A characteristic feature of tricyclic antidepressants is their ability to inhibit the uptake of noradrenaline (NA) and 5-hydroxytryptamine (5-HT). Secondary tricyclics, such as desipramine, have a greater inhibitory effect on NA uptake whereas tertiary tricyclics, for example chlorimipramine, preferentially block 5-HT uptake. Carlsson & Lindqvist (1978) have found a correlation between blockade of transmitter uptake and transmitter synthesis following the acute administration of a number of established and potential antidepressants. To be clinically effective, antidepressants must be administered chronically. Hence the synthesis