The influence of hypothermia on chlorpromazine-induced metabolic changes in mouse heart and brain

Disturbances in cardiac rhythm and metabolism produced by chlorpromazine have been noted in the whole animal (Huston & Bell, 1966), and in the isolated heart (Prindle, Gold & others, 1970) and Alexander & Nino (1969) were sometimes able to relate these disturbances with changes in mitochondrial structure. Chlorpromazine also decreases the incorporation of isotopically-labelled glucose into brain protein (Skinner & Spector, 1968). Furthermore, chlorpromazine is known to interfere with the regulation of body temperature (Lessin & Parkes, 1957). In view of these findings, together with the relevance of establishing the cardiac effects of chlorpromazine, we have compared the effects of chlorpromazine on total [^{14}C]glucose uptake and incorporation into the protein of heart and brain of mice; the significance of chlorpromazine hypothermia in explaining these results has also been investigated.

The experiments were done at 21° and at 38°, rectal temperatures were recorded at 10 min intervals with a thermistor probe. The experiments were made at the same time each day.

Adult female SAS/ICI albino mice, 25–40 g, had, by intraperitoneal injection, 0·9% saline, 5 ml/kg, or chlorpromazine, 20 mg/kg. Thirty min after the first injection the mice were given 5μCi[U-^{14}C]-D-glucose in 0·1 ml saline (3 mCi/mmol Radiochemical Centre, Amersham) intraperitoneally. The animals were killed by cervical dislocation 30 min after the isotope injection and the heart and cerebral hemispheres rapidly removed, washed in ice-cold saline for 2 min, blotted dry and weighed. The tissues were homogenized in 3 ml of ice-cold 10% trichloroacetic acid and then chemically fractionated (Vrba, Gaitonde & Richter, 1962).

The protein precipitate was thrice washed in trichloroacetic acid and the supernatant retained. Lipids were extracted with three 2 ml volumes of acetone. The residual precipitate was hydrolysed with 1·5 ml of 0·3M potassium hydroxide at 37° for at least 24 h and the final solution was cleared with 1·5 ml of M Hyamine-10-X in methanol. The radioactivity of the samples was measured in a Beckmann automatic liquid scintillation counter. Total uptake was expressed as counts min^{-1} mg^{-1} and incorporation into protein as a percentage of the total uptake. The results for the cerebral hemispheres were examined by an analysis of variance based on that described by Snedecor (1962) for hierarchal classifications. The results for the hearts were analysed by Students *t*-test; where variances were incompatible, a modified *t*-test was used. From the analyses it was concluded that the errors introduced by the experimental technique were less than 5%. The percentage recovery from crude homogenate in seven experiments was 97·3 $\pm$ 5·44% (mean $\pm$s.e.). Further extraction of lipid with ethanol–ethyl ether (3 : 1 v/v); chloroform–methanol (2 : 1 v/v) and ether showed that acetone failed to extract 6·3% (mean of 4) and 13·1% (mean of 7) of the total lipid from heart and brain respectively.

A dose of chlorpromazine, 20 mg/kg, reduced the mean body temperature from 37·4° to 29·2° (n = 16). In the experiment in which body temperature was maintained the mean temperatures of the test and control animals were 37·88° (n = 70) and 37·83° (n = 70) respectively.

The total uptake and percentage incorporation of ^{14}C into protein for hypothermic and normothermic mice are shown in Table 1. There was no significant change in total uptake or incorporation into protein of heart tissue after administration of chlorpromazine in either hypothermic or normothermic mice. However, a significant reduction in the percentage incorporation into cerebral protein ($P < 0{\cdot}001$) was demonstrated in hypothermic chlorpromazine-treated mice. It is therefore possible

Table 1. *The effect of chlorpromazine on the uptake of radioactivity into mouse heart and brain following administration of* [*U*-^{14}*C*]-D-*glucose*. Results are expressed as counts min^{-1} mg^{-1} wet weight and as percentage incorporation of radioactivity into protein.

	Hypothermic				Normothermic			
	Cpz	Control	Difference ±95% limits	*P*	Cpz	Control	Difference ±95% limits	*P*
	Cerebral hemispheres							
Total uptake counts mg^{-1} mg^{-1}	463	563	100 ± 193	N.S.	737	791	54 ± 38	N.S.
% incorporation into protein	1·74	3·44	1·69 ± 0·92	0·001	2·90	3·47	0·57 ± 0·83	N.S.
	Hearts							
Total uptake counts min^{-1} mg^{-1}	259	364	105 ± 107	N.S.	223	341	118 ± 141	N.S.
% incorporation into protein	4·39	4·04	0·35 ± 4·01	N.S.	7·40	5·66	1·74 ± 2·35	N.S.

Cpz = Chlorpromazine-treated animals
N.S. = Non-significant at $P = 0·05$

that there is a relation between the decreased incorporation of radioactivity into brain protein and the fall in body temperature induced by chlorpromazine. Thus the effect of chlorpromazine in decreasing the rate of cerebral protein synthesis may not be a primary effect on protein synthesizing mechanisms but could be secondary to the hypothermia induced by the drug. The results in Table 1 demonstrate that the inhibition of ^{14}C incorporation into cerebral protein by chlorpromazine is, to a large degree, dependent upon the hypothermia induced by this drug, although the total glucose uptake is not significantly affected. In the normothermic chlorpromazine-treated mice no significant decrease in the incorporation of ^{14}C into cerebral protein over the corresponding controls was noted. Similar results have been reported concerning the inhibition of brain protein synthesis from labelled amino-acids by chlorpromazine (Shuster & Hannam, 1964). Furthermore, it has been demonstrated that chlorpromazine does not inhibit the cerebral synthesis of amino-acids from glucose carbon in rats whose body temperature was maintained (Bachelard, Gaitonde & Vrba, 1966), whereas in hypothermic animals there is a decreased incorporation into α-keto-acids and α-amino-acids (Bachelard & Lindsay, 1966). It is important to note however that chlorpromazine has been shown to inhibit protein synthesis in rat brain slices maintained *in vitro* at 37° (Lindan, Quastel & Sved, 1957).

The absence of an inhibitory effect on cardiac protein synthesis by chlorpromazine is interesting because it may represent a difference in specificity of drug action-between these two excitable tissues. This is consistent with observations made on rats in which there is no effect of chlorpromazine on amino-acid synthesis from glucose by the hearts of temperature-maintained animals (Bachelard & others, 1966).

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Effects of small doses of haloperidol on timing behaviour

The depressive effects of drugs of the phenothiazine and butyrophenone group are thought to be connected with the inability to block post-synaptic receptors in the central catecholamine neurons (see Andén, Carlsson & Häggendal 1969). Clinically, small doses of some phenothiazines—especially those with a propylpiperazine side chain—and some butyrophenones, e.g. triperidol, appear to have certain stimulating properties (Di Mascio, Havens & others 1961, Lingjaerde 1966). We have observed, and others have reported (Janssen, 1962; Monti & Hance, 1967) the butyrophenone derivative haloperidol to have some stimulant action in small doses.

Four male Sprague-Dawley rats were food-deprived and kept at 80% of their free-feeding weight (278 $\pm$ 6 g). The rats were trained to press a lever in standard behavioural chambers (Model E3125A, Grason-Stadler) to get food pellets (Noyes, 45 mg) on a DRL-20 schedule (Differential Reinforcement of Low rates), whereby a depression of the lever produced the pellet only if it followed the preceeding lever depression by at least 20 s. Every premature lever press (<20 s after the last response) starts the interval again.

The Inter-Response Times (IRT, interval between successive responses) were divided in 3 s categories: 0–2, 3–5, etc. Presses spaced more than 30 s apart were collected in a last category. Leverpress responses were recorded on digital counters and categorized automatically. For each session a mean IRT was calculated. The distribution was symmetrically cut (below 9–12 and above 27–30) around the optimal reinforced IRT (18–21), to avoid an open interval. A grand mean for the control and the different treatments was calculated and a 98% confidence interval determined for the differences between the means (Scheffé, 1959).

Each rat was exposed to daily sessions for 21–22 consecutive days. Experimental sessions were separated by two control sessions. The complete sessions consisted of 15 min adaptation, immediately followed by 60 min, in which responses were recorded. 15 min before the start of the experimental sessions, animals were injected with haloperidol 0·01, 0·02 or 0·03 mg/kg: each dose was tested twice on each rat. No injections were made before any of the control sessions. Drugs were freshly prepared and injected intraperitoneally in a volume of 2 ml/kg.

The effects of the doses of haloperidol on the behaviour variable used are shown in Table 1. After injection of 0·02 mg/kg of haloperidol the IRT distributions showed a statistically significant ($P < 0·02$) increase in the frequency of short IRTs compared to those in the control distributions. That is, the animals pressed the lever more frequently before the required interval had elapsed. The injections of the other doses of haloperidol, 0·01 and 0·03 mg/kg did not significantly alter the IRT distributions. As assessed by gross observation all the animals displayed a normal behaviour.