Br. J. Pharmac. (1987), **90**, 745–751

Neuroleptic-induced hypothermia in mice: lack of evidence for a central mechanism

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1 The present study investigated the ability of neuroleptic drugs to induce hypothermia in mice when they were administered intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.).

2 Twelve neuroleptics belonging to five chemical classes including phenothiazines, butyrophenones, benzamides, thioxanthenes and diphenylbutylpiperidines were injected i.p. All of them, except benzamides, induced a dose-dependent decrease in rectal temperature.

3 Neuroleptics were administered i.c.v. via cannulae previously implanted in mice to determine whether this response might have a central origin. None of the drugs tested induced hypothermia at doses which did not produce toxic effects. These negative results suggest that neuroleptics act to elicit hypothermia via a peripheral, rather than a central mechanism.

4 Since some neuroleptics possess α-adrenolytic properties which could induce hypothermia by promoting vasodilatation, we attempted to antagonize the hypothermia produced by peripheral administration of two neuroleptics with phenylephrine, an α-adrenoceptor agonist that does not cross the blood-brain barrier. The hypothermia induced by both chlorpromazine and haloperidol was attenuated by phenylephrine, supporting the view that peripheral α-adrenoceptors may mediate neuroleptic-induced hypothermia.

Introduction

It is now well documented that the neuroleptic drugs affect thermoregulation in animals differently, according to the method of administration, the use or absence of restraint and the ambient temperature at which the experiment is carried out. In two very interesting and useful reviews (Clark, 1979; Clark & Lipton, 1985), the results of numerous studies concerning the thermoregulatory action of neuroleptics are quoted and indicate that the systemic injection of these dopamine antagonists generally elicits hypothermia in man and in animals exposed to normal room temperature. The phenothiazines are among the most widely used neuroleptics in the treatment of psychiatric disorders. It is interesting to note that the thermoregulatory action of these agents not only gives rise to adverse side-effects but that it has also been directly exploited in therapy (Hoagland & Bishop, 1961).

The hypothalamus is generally considered to be the most probable site of action of neuroleptics in producing a decrease in temperature. However, the literature contains a number of conflicting accounts of the thermoregulatory effects of centrally-administered neuroleptics. The majority of results indicate that systemic administration of neuroleptics cause hypothermia in rats and in mice (Witherspoon *et al.*, 1957; Lin *et al.*, 1979) whereas intracerebral injection into preoptic nuclei of rats either does not affect rectal temperature (Cox *et al.*, 1981; Colboc *et al.*, 1983) or induces hyperthermia (Rewerski & Jori, 1968; Kirkpatrick & Lomax, 1971; Lin *et al.*, 1982). The frequent clinical use of neuroleptics and the fact that they are known to induce hypothermia in man would suggest that it is highly desirable to know something of the origin of this effect. We have therefore studied a series of neuroleptics, representing each of the major chemical classes, for their ability to induce hypothermia when administered intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) to mice. Mice were used because they are particularly sensitive to the hypothermic action of drugs and because little or no information regarding the thermoregulatory effects of neuroleptics given intracerebrally is available for this species.

An attempt was made to antagonize the hypothermia induced by these neuroleptics. Some neuroleptics possess adrenoceptor blocking properties (Thoenen *et al.*, 1965; Bartholini *et al.*, 1976) which could be responsible for the induction of the hypothermia. Hence, the effects of an α-adrenoceptor agonist on this hypothermia were investigated.

Methods

Animals

Male OF1 mice (Iffa Credo), 7 weeks old and weighing 30–32 g on the day of the experiment, were used. The animals were maintained on a 12 h light-dark cycle (08 h 00 min–20 h 00 min) in a temperature-regulated room (22 ± 1°C). Each mouse was used only once.

Injections

Peripheral injections were given intraperitoneally (i.p.) in a volume of 0.1 ml 10 g^{-1} body weight.

Central injections were made after a guide cannula had been implanted into the right lateral ventricle using a method described previously (Boschi *et al.*, 1981). One week later, the drugs were injected in a volume of 0.5 to 1 µl over a period of 50 to 100 s. Following the experiments, the location of the cannula was verified histologically after an injection of methylene blue into the cannula.

Control animals received the same volume of vehicle alone.

Measurement of rectal temperature

The rectal temperature was measured with a thermocouple probe (Bailey Instruments), carefully inserted to a depth of 1.5 cm. Readings were taken before (60, 30, 0 min) injection and after injection. The post-injection measurement times varied according to the injection route used. Mice were kept in groups of 6 and were free to move in their cages except during the brief time required for temperature measurement. Experiments were carried out between 10 h 00 min and 14 h 00 min.

Drugs

Thioproperazine methane sulphonate (Specia), *cis*-(z)-flupenthixol dihydrochloride (Lundbeck) and phenylephrine (Chibret) were dissolved in water. In the interaction studies, phenylephrine was injected separately from the other drugs. Chlorpromazine hydrochloride (Specia) and haloperidol (10 µg) (Janssen) were dissolved in water containing 1% acetic acid. Commercially available solutions were used to prepare solutions in water of: haloperidol (5 µg) (Haldol, Janssen, containing methyl and propyl parahydroxybenzoate and lactic acid), droperidol (Droleptan, Janssen, containing methyl and propyl parahydroxybenzoate and lactic acid), pimozide (Orap, Janssen, containing methyl and propyl parahydroxybenzoate and tartic acid), sulpiride (Dogmatil, Delagrange), sultopride (Barnétil, Delagrange, containing benzylic alcohol), levomepromazine (Noziman, Spécia, containing ascorbic acid), pipotiazine (Piportil, Spécia, containing methane sulphonic acid and ascorbic acid), prochlorperazine (Témentil, Spécia, containing citric acid, saccharine and ethylic alcohol) and perimetazine (Leptryl, Roger-Bellon, containing sodium citrate and sodium metabisulphite). Control experiments were carried out with drug vehicles in the i.p. and i.c.v. experiments. Doses of drugs are expressed as the weight of the salt.

Statistics

The data were analysed by means of a one-way analysis of variance (Kruskall-Wallis) and subsequently with the Mann-Whitney U test.

Results

Effects of peripheral administration of neuroleptics on the rectal temperature of mice

Five chemical classes of neuroleptics including phenothiazines, butyrophenones, benzamides, thioxanthenes and diphenylbutylpiperidines were examined. Figure 1 shows the acute effects of the twelve neuroleptic compounds when administered intraperitoneally to mice. It can be seen that all of them, except the benzamides (sulpiride and sultopride), caused a dose-dependent decrease in the rectal temperature. The phenothiazines produced the strongest hypothermia. Chlorpromazine, when administered systemically (5 mg kg^{-1} i.p.), induced a marked fall in rectal temperature 2 h after the injection (−5.10 ± 0.43°C). Even a smaller dose (2.5 mg kg^{-1}) had a strong hypothermic effect (−3.48 ± 0.6°C). The responses to levomepromazine were very similar. The other neuroleptic drugs decreased the rectal temperature to a lesser extent. Both sultopride and sulpiride did not modify the rectal temperature at doses of 50 and 100 mg kg^{-1} i.p. The latter produced hypothermia only at a dose (200 mg kg^{-1} i.p.) inducing toxic effects (rigidity, convulsions and sometimes death).

Effects of intracerebral administration of neuroleptics on the rectal temperature of mice

The drugs were injected directly into the cerebral ventricles in an attempt to elucidate whether they might exert their thermoregulatory action by a central mechanism. Surprisingly, none of the neuroleptics tested was able to induce hypothermia in mice after intracerebroventricular (i.c.v.) injection at doses which are devoid of any toxic effects (Figure 2). It is generally agreed that the i.p./i.c.v. dose-ratio must be about 1000 for there to be any possibility of the drug acting centrally. The main results obtained are sum-

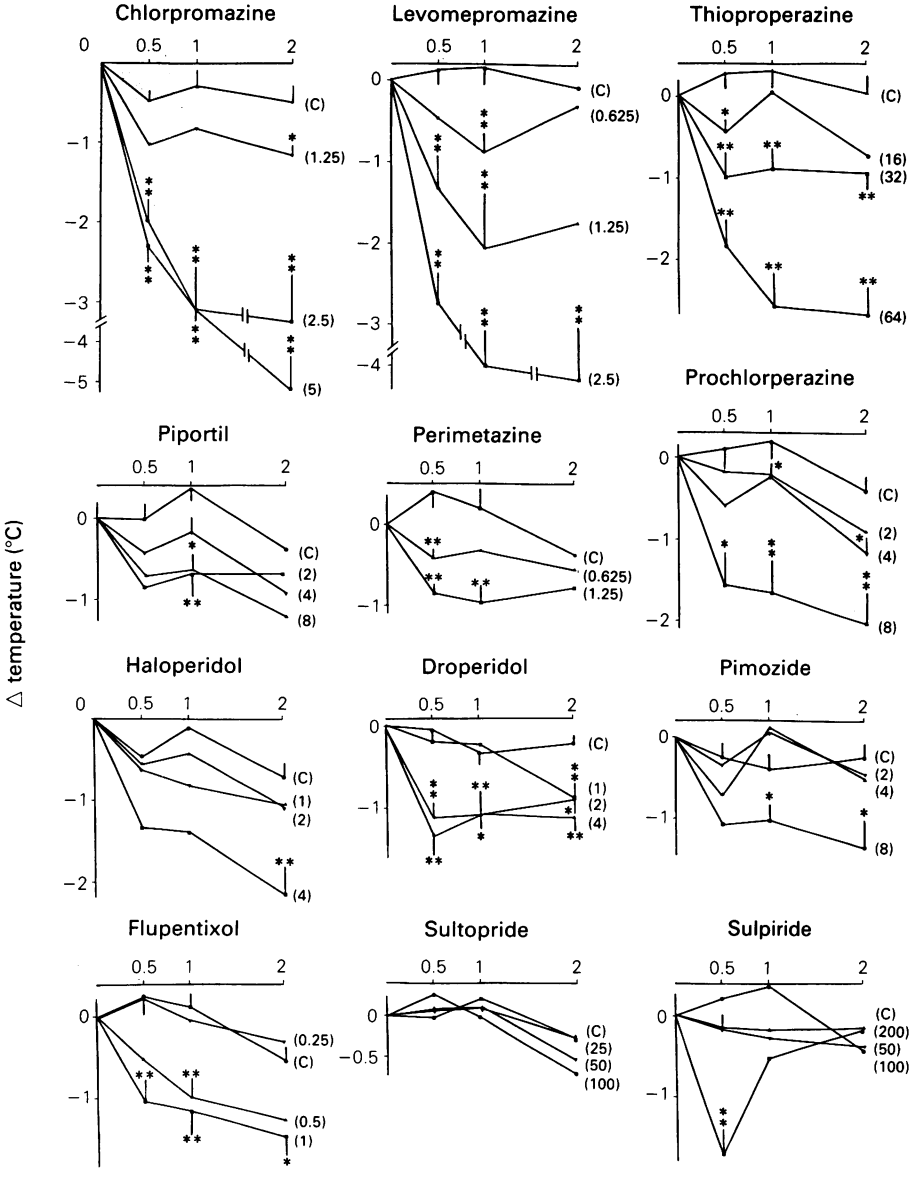

Figure 1 Time course of the change in rectal temperature (°C) in mice produced by increasing doses of neuroleptic drugs given i.p. The effect of neuroleptics is expressed as the difference between pre- and post-injection temperature (ordinate scales). Each point represents the mean of 7 to 8 mice. In order to simplify the figure, the vertical lines indicating the s.e.mean are shown only for the significant values. The values obtained for the treated and control animals were compared using a one-way analysis of variance (Kruskall-Wallis) and, subsequently, the Mann-Whitney U test: $*P<0.05$; $**P<0.01$. (C) Control responses. Numbers in parentheses: doses of neuroleptics in $mg\,kg^{-1}$. Abscissa scales: time in h.

marized in Table 1. Thus, for phenothiazines, the smallest hypothermic dose of chlorpromazine ($2.5\,mg\,kg^{-1}$) given i.p. caused a strong hypothermia while the i.c.v. administration of a dose of $10\,\mu g$ was without effect. A slight transient hypothermia occurred only with a large dose of $20\,\mu g$ (a dose-ratio of 125). The other phenothiazines tested did not produce a hypothermic response when given i.c.v.

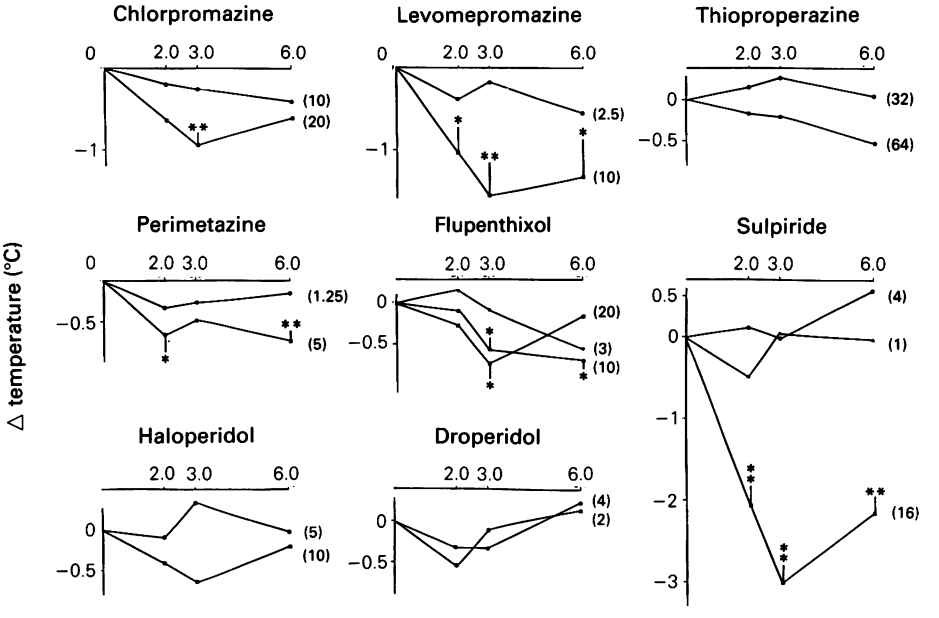

Figure 2 Time course of the change in rectal temperature (°C, ordinate scales) in mice produced by increasing doses of neuroleptic drugs given i.c.v. The effects of the neuroleptics are expressed as the difference between pre- and post-injection temperature less the effect of vehicle alone (controls). Each point represents the mean of 7 to 11 mice derived from 2 experiments. In order to simplify the figure, the vertical lines indicating the s.e.mean shown only for the significant values. The values obtained for the treated and control animals were compared using the Mann-Whitney U test: $*P<0.05$; $**P<0.01$. Numbers in parentheses: doses of neuroleptics in μg. Abscissa scales: time in min.

except at high doses. The butyrophenones (haloperidol and droperidol) produced similar responses. The ratio between the smallest doses of the thioxanthene cis-(Z)-flupentixol given i.p. and i.c.v. was 50. The effect was not greater with 20 μg than with 10 μg of flupentixol. The same i.p. and i.c.v. response pattern was seen for the benzamides. Doses of sulpiride given i.c.v. were without effect on rectal tem-

Table 1 Effects of some neuroleptics administered by i.p. and i.c.v. route on the rectal temperature of mice

Drugs	*Doses*	*I.p./i.c.v. dose-ratios*	*Decrease in rectal temperature°C* (mean ± *s.e.mean*)
Chlorpromazine	2.5 mg kg^{-1} i.p.	125	−3.48 ± 0.6
	20 μg i.c.v.		−0.94 ± 0.14
Levomepromazine	1.25 mg kg^{-1} i.p.	500	−2.05 ± 0.68
	2.5 μg i.c.v.		No effect
Thioproperazine	32 mg kg^{-1} i.p.	500	−0.99 ± 0.31
	64 μg i.c.v.		No effect
Perimetazine	1.25 mg kg^{-1} i.p.	1000	−0.95 ± 0.23
	1.25 μg i.c.v.		No effect
Haloperidol	4 mg kg^{-1} i.p.	500	−2.16 ± 0.35
	10 μg i.c.v.		No effect
Droperidol	2 mg kg^{-1} i.p.	400	−1.34 ± 0.25
	4 μg i.c.v.		No effect
Flupenthixol	0.5 mg kg^{-1} i.p.	50	−1.12 ± 0.23
	10 μg i.c.v.		−0.58 ± 0.16

perature and a toxic dose (which produced rigidity) of 16 μg was needed to obtain a hypothermic response.

Lack of solubility prevented i.c.v. testing of the other neuroleptics.

Effect of an α-adrenoceptor agonist on the hypothermia induced by neuroleptics in mice

Since most neuroleptics possess α-adrenolytic properties (see Introduction), we attempted to antagonize the hypothermia induced by peripheral administration of neuroleptics with phenylephrine, a drug which activates peripheral α-adrenoceptors. Two neuroleptics from different chemical groups were chosen: the potent hypothermic phenothiazine, chlorpromazine, and the classical butyrophenone, haloperidol. As illustrated in Figure 3a, the concomitant administration of phenylephrine (6 mg kg^{-1} i.p.), at a dose which was without effect on rectal temperature, and chlorpromazine (2.5 mg kg^{-1} i.p.) totally suppressed the hypothermia at 30 min and significantly reduced it at 1 and 2 h. Similarly, the simultaneous injection of phenylephrine (6 mg kg^{-1} i.p.) and haloperidol (4 mg kg^{-1} i.p.) suppressed the hypothermia at 30 min (Figure 3b).

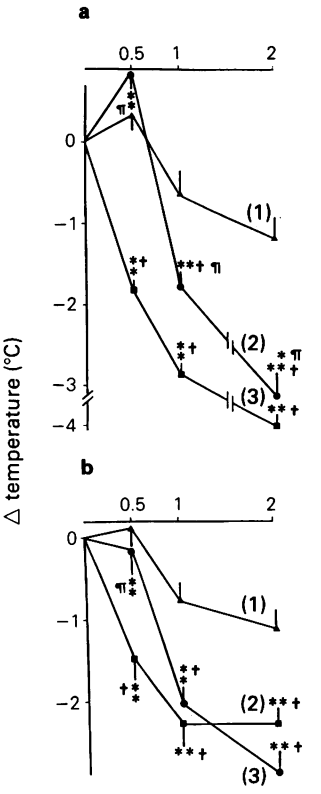

Figure 3 Time course of the change in rectal temperature (°C) in mice produced by a combination of phenylephrine and chlorpromazine (a):(1) water (i.p.) and phenylephrine (6 mg kg^{-1} i.p.); (2) chlorpromazine (2.5 mg kg^{-1} i.p.) and phenylephrine (6 mg kg^{-1} i.p.); (3) chlorpromazine (2.5 mg kg^{-1} i.p.) and water (i.p.), and a combination of phenylephrine and haloperidol (b): (1) water (i.p.) and phenylephrine (6 mg kg^{-1} i.p.); (2) haloperidol (4 mg kg^{-1} i.p.) and phenylephrine (6 mg kg^{-1} i.p.); (3) haloperidol (4 mg kg^{-1} i.p.) and water (i.p.). The drugs were given simultaneously but separately. Each point represents the mean of 9 to 10 mice. Vertical lines represent s.e.mean. † Compared with the group treated by phenylephrine + water, ¶ compared with the group treated by the neuroleptic + water, $*P<0.05$; $**P<0.01$, Kruskall-Wallis and Mann-Whitney U tests.

Discussion

The sites and mechanisms of the thermoregulatory action of neuroleptic drugs are still largely unknown. In the present study, we have investigated the action of neuroleptics on the rectal temperature after intracerebral administration. Although neuroleptics are generally administered peripherally, the hypothermic effect has been said to involve the central nervous system, and more precisely the hypothalamus, since this area plays an important role in temperature regulation (Cox, 1979). However, the few data which provide information regarding hypothermia produced by cerebral administration of neuroleptics are controversial. Several studies have shown that intrahypothalamic injection of neuroleptics produces an opposite effect in rats, i.e. hyperthermia (Rewerski & Jori, 1968; Kirkpatrick & Lomax, 1971; Lin *et al.*, 1982) or no effect (Cox *et al.*, 1981; Colboc *et al.*, 1983). The present results in the mouse are in accord with these findings since all the neuroleptics administered intracerebroventricularly were unable to elicit a real hypothermia, although their systemic administration easily produced hypothermia. We cannot explain this lack of effect by the use of inadequate doses of neuroleptics. If these drugs act by a central mechanism, their intracerebroventricular injection should induce at least a similar decrease of temperature. In fact, the doses injected by the i.c.v. route were ineffective and higher doses produced just a slight hypothermia with occasionally concomitant toxic effects. The hypothermia observed in these cases was always much smaller than that obtained by i.p. administration: −0.94°C after 20 μg i.c.v. compared to −3.48°C after 2.5 mg kg^{-1} i.p. for chlorpromazine. One possible explanation for this difference is that the neuroleptics are poorly absorbed into parenchyma of

the brain after intraventricular administration. But, this is not consistent with the high lipophilicity of these drugs. Furthermore, the hypothalamus, which is the likely candidate for the hypothermic effect of neuroleptics, is located close to the ventricular system. We have also studied the hypothermia induced by dexamphetamine. This compound is less lipophilic than neuroleptics but nevertheless, it readily produced a strong hypothermic response after i.c.v. injection as well as after administration to certain specific brain areas (Boschi & Rips, 1982). Another explanation is that the hypothermic effect of neuroleptics may possibly be mediated at a cerebral site outside the central nervous system, such as the median eminence. However, neuroleptics readily enter the brain after systemic administration. Moreover, if the median eminence was involved in mediating this hypothermia it seems unlikely that neuroleptics given i.c.v. would not diffuse from the lateral ventricle to the median eminence via the third ventricle. Finally, the hypothermia produced by systemic injection of neuroleptics may be mediated via the peripheral nervous system. A quaternary derivative of chlorpromazine (Cpz), N-methyl Cpz, is thought not to pass the blood-brain barrier (Hansson & Schmiterlöw, 1961). This compound caused a potent hypothermia in rats maintained at normal ambient temperature (Kirkpatrick & Lomax, 1971). The authors concluded that the hypothermic effects of both N-methyl-Cpz and Cpz are mediated at peripheral sites. Behavioural studies have been used to ascertain whether Cpz acts centrally. The review by Borbely & Loepfe-Hinkkanen (1979) provides no evidence to indicate that the hypothermia induced by phenothiazines in rodents is a consequence of a change in central regulatory mechanisms or a change in the setpoint. In contrast, Cpz clearly induced peripheral vasodilatation which could constitute a major factor in the lowering of body temperature (Borbely & Loepfe-Hinkkanen, 1979). These findings, together with the negative results obtained after central administration of neuroleptics in the present study, suggest that neuroleptics are acting to elicit hypothermia via a peripheral rather than a central mechanism.

A survey of the available data indicated that activation of dopamine receptors within the central nervous system can bring about thermoregulatory changes. Thus, central injection of dopamine or other direct dopamine agonists produced hypothermia in a wide variety of species (Cox, 1979). From studies of drugs which mediate their effects through dopamine release (such as amphetamine), some evidence has been obtained indicating that endogenous dopamine is involved in the hypothermic response (Boschi & Launay, 1985). The use of specific antagonists has indicated an action on central dopamine receptors as the most likely explanation of the observed effects (Cox, 1979).

However, in a recent study, Feigenbaum & Yanai (1985) suggested that dopamine receptors do not mediate hypothermia *per se*, but rather that this hypothermia is more complex, involving other factors. Until now, the mechanisms generally involved in the hypothermia induced by neuroleptics are believed to be dopaminergic. If dopamine receptors do mediate hypothermia induced by neuroleptics were believed to be dopaminergic. If dopamine receptors do mediate they do the opposite. Another argument in favour of the suggestion that dopamine receptors do not mediate the hypothermia induced by neuroleptics is the finding that, in our experimental conditions, benzamides did not affect the rectal temperature in mice following both i.c.v. and i.p. administration. Benzamides have been described as selective dopamine receptor antagonists, unlike other neuroleptics (Jenner & Marsden, 1981).

It is now well established that neuroleptics block neurotransmitter receptors other than dopamine receptors. Neuroleptics, such as chlorpromazine, pimozide, haloperidol have been shown to block α-adrenoceptors in various structures of the central (Arbilla *et al.*, 1978; Bartholini *et al.*, 1976) and peripheral nervous system (Thoenen *et al.*, 1965; Hope *et al.* 1978). This antagonistic action of neuroleptics could induce hypothermia by promoting vasodilatation. Phenylephrine, the α-adrenoceptor agonist used in this study to antagonize the hypothermia induced by the neuroleptics, has a short duration of action and does not cross (or very little) the blood-brain barrier (Weiner, 1985). The fact that chlorpromazine- and haloperidol-induced hypothermia was abolished 30 min after simultaneous injection of phenylephrine, is in agreement with the view that peripheral α-adrenoceptors may mediate the hypothermia induced by neuroleptics.

We are grateful to Mrs C. Souriau for her invaluable help in the preparation of the material used for intracerebral injections. We also thank the pharmaceutical firms for their generous gifts of the compounds mentioned in the methods section.

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(Received September 1, 1986.
Revised November 14, 1986.
Accepted December 15, 1986.)