EFFECTS OF CHLORPROMAZINE ON LIPOLYSIS IN BROWN AND WHITE ADIPOSE TISSUES OF COLD-EXPOSED RATS

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Abstract—Effects of chlorpromazine (CPZ) on lipolysis in brown (interscapular: IBAT) and white (epididymal: EWAT) adipose tissues in cold-exposed rats were studied in vitro. Adult male Wistar-Imamichi rats were exposed to 4°C for 1 (acute cold-exposed) or 30 (cold-acclimated) days. The spontaneous free fatty acid (FFA) release from interscapular brown adipose tissue (IBAT) of control rats, was three fold higher than epididymal white adipose tissue (EWAT). FFA release from the EWAT of acute cold-exposed rats was significantly higher than that of control rats. After cold acclimation, FFA release from IBAT increased up to three times that of the control level. CPZ (10⁻¹, 10⁻³ M) suppressed the elevated lipolytic activity in IBAT of cold-acclimated rats. However CPZ had no effect on FFA release from EWAT or IBAT in all other groups. A single intravenous administration of 10 mg/kg 6-hydroxydopamine (6-OHDA) produced a marked decrease in the concentration of noradrenaline and dopamine in IBAT and EWAT of cold-acclimated rats. FFA release from IBAT in 6-OHDA treated, cold-acclimated rats decreased to 50% of untreated, cold-acclimated rats. A further decrease in lipolytic activity in the IBAT of 6-OHDA treated, cold-acclimated rats was produced following CPZ treatment. CPZ also depressed the lipolysis stimulated by the added noradrenaline (3 x 10⁻⁵ M). These results suggest that the effect of CPZ on the lipolysis in the IBAT from cold-acclimated rats is mediated by a modification of sympathetic activity.

It is now generally accepted that the sympathetic nervous system plays an important role in the mobilization of free fatty acids (FFA) from adipose tissues. In particular, brown adipose tissue which is hypertrophied in cold-acclimated animals (1, 2) is intensively innervated with sympathetic nerves in comparison to the white adipose tissue (3). The function of brown adipose tissue is to produce heat responding to the cold-stimulated release of noradrenaline from sympathetic nerve endings within the tissue (4–6). Free fatty acids following the augmented lipolysis may serve as essential substrates for thermogenesis in cold environments (7). A number of agents have been shown to inhibit lipolysis by interfering with steps in the lipolytic process. Previous work has shown that chlorpromazine (CPZ) inhibits noradrenaline induced in vitro lipolysis in the rat epididymal fat (8). In vivo, CPZ also increases plasma FFA level (9, 10).

We previously found that removal of interscapular brown adipose tissue (IBAT) potentiated the hypothermia induced by CPZ injection in the cold-acclimated rats (11). This finding indicated that IBAT is involved in the CPZ induced hypothermia in the cold-acclimated rat. Therefore, the following experiments were undertaken to determine the effects of CPZ on the stimulated lipolysis of adipose tissue which occurs during the cold
exposure. Effects of chlorpromazine on the in vitro lipolysis were studied in the brown and white adipose tissues from control, acute cold-exposed and cold-acclimated rats.

MATERIALS AND METHODS

Animals: Adult male Wistar-Imamichi rats were continuously exposed to 4°C for 1(1d) or 30 days (30) and the control animals (0d) were housed at regulated room temperature (23±1°C). During these periods the animals were fed a rat standard diet (labo MR bleeder from Nihonnohsankogyo Co. Ltd.) and provided water ad libitum. Each animal in the cold room (4°C) was housed in an individual cage. Some animals were given 10 mg/kg/5 ml 6-hydroxydopamine (6-OHDA) i.v.. The 6-OHDA was dissolved in 0.9% NaCl solution containing 0.5% ascorbic acid immediately before injection.

Incubations: After decapitation, IBAT and EWAT were quickly excised and cut into four pieces of 15 mg each and placed in each tube containing 3 ml incubation medium. Krebs-Ringer phosphate buffer, pH 7.4, containing 2% bovine serum albumin (Armour, fraction V), equilibrated with oxygen gas, was used as the incubation medium. After a preliminary incubation at 37°C for 20 min, CPZ in various concentrations (10⁻⁵, 10⁻⁴ and 10⁻³ M) and/or noradrenaline (3 x 10⁻⁵ M) were added and the incubation was continued further for 120 min at 37°C under oxygen gas with shaking at 60 oscillations per min.

Assays: At the end of the incubation, free fatty acid released into the incubation medium was determined colorimetrically by the method of Novák (12). Adipose tissue lipase was assayed by the method of Rizack (13), measuring the FFA formed from ediol (a commercial emulsion of 50% coconut oil). The enzyme activity was expressed as μEq of free fatty acid/g of adipose tissue/hr. Catecholamines (noradrenaline: NA and dopamine: DA) in adipose tissue were assayed by the method of Ansell and Beeson (14).

Reagents and Drugs: Noradrenaline bitartrate and 6-hydroxydopamine hydrobromide were from Sigma Chemical Co., bovine serum albumin (fraction V) was from Armour Pharmaceutical Co., Ediol was from Calbiochem. and chlorpromazine hydrochloride was a gift from Shionogi Pharmaceutical Co. Ltd.. All other chemicals were of analytical grade.

RESULTS

The basal lipolysis in brown and white adipose tissue of control (0d), acute cold-exposed (1d) and cold-acclimated (30d) rats is shown in Fig. 1. A spontaneous FFA release of the IBAT from control rats (0d) was three fold higher than that of the EWAT. After cold acclimation, FFA release from the IBAT increased up to three times that of the control level, whereas FFA release remained unchanged in the IBAT of acute cold-exposed rats. FFA release from the EWAT of acute cold-exposed rats was significantly higher than that in the control rats. But this elevated release was restored to the normal level after cold acclimation. The effect of CPZ on the basal lipolysis in fragments of two kinds of adipose tissues (IBAT, EWAT) in control (0d), acute cold-exposed (1d) and cold-acclimated (30d) rats is shown in Fig. 2. A significant change was demonstrated following CPZ addition only in the IBAT of cold-acclimated rats, whereas no change in lipolytic activity was induced.
FIG. 1. Basal lipolysis in brown (interscapular: IBAT) and white (epididymal: EWAT) adipose tissues of control (0d), acute cold-exposed (1d) and cold-acclimated (30d) rats. Lipolytic activity was expressed as μEq free fatty acid/g of adipose tissue/120 min. Each value represents the mean±standard error. Figures in parentheses indicate the number of determinations. P values were calculated by Student’s t-test. **: P<0.01, ***: P<0.001.

FIG. 2. Effects of chlorpromazine in the basal lipolysis in brown (interscapular: IBAT) and white (epididymal: EWAT) adipose tissues in control (0d), acute cold-exposed (1d) and cold-acclimated (30d) rats. Samples of IBAT and EWAT (15 mg x 4) were incubated in 3 ml of KRP buffer pH 7.4 containing 2% bovine serum albumin at 37°C for 120 min. After a preincubation of 20 min, CPZ (10⁻⁵, 10⁻⁴, or 10⁻³ M) or incubation medium was added in 0.3 ml amounts to each tube. Each value represents the mean±standard error. Figures in parentheses indicate the number of determinations. P values were calculated by Student’s t-test. ***: P<0.001.
either in the IBAT of the other groups (0d, 1d) or in the EWAT of all groups. The inhibitory
effect of CPZ on the spontaneous FFA release from the IBAT of cold-acclimated rats was
64% at 10^{-3} M, 44% at 10^{-4} M but was not observed in a dose of 10^{-5} M.

Since we could demonstrate the effect of CPZ only with the cold-acclimated rats, the
following experiments were done using the cold-acclimated animals. To determine the
contribution of sympathetic activity on the lipolysis in the IBAT of cold-acclimated rats,
the next experiment (Fig. 3) was carried out using 6-OHDA treated rats. Noradrenaline
contents in IBAT and EWAT of control rats (0d) were 1.39±0.13 and 0.04±0.01 μg/g,
respectively. After cold acclimation, noradrenaline level in the IBAT was elevated (1.85
±0.17 μg/g), but was not significantly modified by cold exposure for 1 day. On the other
hand, no change in noradrenaline level in the EWAT was noted either in the cold-acclimated
(30d) or the cold-exposed (1d) rats. As demonstrated in Fig. 3A, after 6-OHDA adminis-
tration, noradrenaline contents were significantly decreased both in the IBAT and in the
EWAT of cold-acclimated rats, but the extent of decrease in the IBAT was more marked
than that in the EWAT. The administration of 6-OHDA produced a 50% reduction of
lipolysis in the IBAT from cold-acclimated rats, whereas no change in the EWAT was
observed. All concentrations of CPZ (10^{-5}, 10^{-4} and 10^{-3} M) inhibited significantly FFA
release from the IBAT in 6-OHDA treated, cold-acclimated rats. The inhibitory effect
did not vary with the different concentrations of CPZ. In the EWAT from 6-OHDA
treated, cold-acclimated rats, CPZ (10^{-5}, 10^{-4} and 10^{-3} M) was ineffective for a spontaneous
FFA release.

Figure 4 shows the effects of CPZ on the noradrenaline-induced lipolysis in the IBAT

\[ \text{Fig. 3. A) Catecholamine concentrations (noradrenaline: NA, dopamine: DA) in}
\text{IBAT and EWAT from cold-acclimated rats 4 hr after 6-OHDA 10 mg/kg i.v.}
\text{B) Effects of chlorpromazine on the lipolytic response in IBAT and EWAT from}
\text{6-OHDA treated, cold-acclimated rats. The experimental conditions were as}
\text{described in Fig. 2. Each value represents the mean±standard error. Figures}
\text{in parentheses indicate the number of determinations. P values were calculated}
\text{by Student's t-test. *: P<0.05, **: P<0.01, ***: P<0.001.} \]
and the EWAT of cold-acclimated (30d) rats. In these rats, the effects of added noradrenaline on FFA release in the IBAT were not so marked in comparison with that effects in the EWAT. This seems to indicate the reduced sensitivity to the exogenous noradrenaline in the IBAT of cold-acclimated rats in the response of lipolytic activity. A dose-related inhibition by CPZ of noradrenaline-induced FFA release occurred both in brown and white adipose tissues. This inhibitory effect by CPZ in the IBAT was greater than that in the EWAT.

Figure 5 shows the effects of CPZ on the lipase activity of the IBAT and the EWAT from cold-acclimated rats. The lipase activity of the IBAT was much higher than that of the EWAT. The lipase activity in the IBAT and the EWAT of cold-acclimated rats was
significantly (P<0.001) inhibited by CPZ at a high concentration (10^{-3} M), but not at a concentration of 10^{-4} and 10^{-5} M.

**DISCUSSION**

It was confirmed in this study that there is a high degree of basal lipolytic activity in brown adipose tissue in comparison with the activity in white adipose tissue in control rats. Basal lipolytic activity in brown adipose tissue was markedly elevated by cold acclimation, whereas the activity remained unchanged in white adipose tissue. Such an analogous difference between brown and white adipose tissues of cold-acclimated rats was reported by Dorigo et al. (15, 16) and Bertin (17). Dorigo et al. (15) found the high level of FFA within the cells of brown adipose tissue in normal and cold-acclimatized rats but not within the cells of white adipose tissue and concluded that the high level of FFA in brown adipose tissue cells would allow for a store of the physiological substrate for thermogesesis, thus emphasizing the physiologically important role of brown adipose tissue in thermogenesis. The adrenergic innervation of brown adipose tissue is more intensive than that of white adipose tissue (18). Biochemical investigations have shown that brown adipose tissues from rats contain a higher concentration of noradrenaline (19-20) and a histochemical fluorescence technique has demonstrated a rich supply of noradrenaline-containing fibers (21-23). Moreover, this innervation is more prominent after cold acclimation (24, 25). Derry et al. (22) proposed that there are two types of postganglionic sympathetic fibers within brown adipose tissue.

It is reasonable to assume that such a striking difference of adrenergic innervation between brown and white adipose tissues results in the different lipid metabolism in the two kinds of adipose tissues. Numerous studies have been done on the neuronal control of lipolysis and there is functional evidence for the existence of sympathetic ganglia in the IBAT of the rat, demonstrated by Steiner et al. using the sympathetic ganglionic stimulant dimethylphenylpipеразинум (DMPP) (26). In white adipose tissue, Cantu et al. (27) found no changes in FFA release and glycerol production in vitro, after denervation, and concluded that the effects of denervation on lipid metabolism in white adipose tissue may be secondary to changes in blood flow.

In the experiment herein, FFA release in IBAT from cold-acclimated rats decreased by about 50% 4 hr following the administration of 6-OHDA, whereas no changes were observed in the EWAT (Fig. 3). Thus, the basal lipolytic activity in brown adipose tissue of cold-acclimated rats is, to some extent, dependent on the sympathetic neuronal control, whereas sympathetic neurons may not be primary factors in the in vitro lipolysis of white adipose tissue. Furthermore, the suppression by 6-OHDA of the lipolysis in the IBAT was not complete despite a remarkable reduction in the levels of endogenous noradrenaline (Fig. 3). It was estimated that about 50% of total lipolytic activity is controllable by endogenous catecholamine. Investigations on the exogenous catecholamine-sensitivity of brown and white adipose tissues were carried out by Joel (28) and by Kumon et al. (29) using rats and rabbits, respectively. They obtained a dose-response curve for the effect of catecholamines
on FFA release from adipose tissue and demonstrated that the brown adipose tissue was more sensitive to catecholamine than was the white adipose tissue. In the present work, the responsiveness to $3 \times 10^{-5}$ M noradrenaline of the brown adipose tissue from cold-acclimated rats was remarkably lower than that of the white adipose tissue (Fig. 4). This result is quite different from the response in normal rats but these observations are in agreement with the finding obtained by Dorigo et al. (15). They attributed the reduced lipolytic response to noradrenaline of brown adipose tissue from cold-acclimated rats to the high level of FFA within the cells, which produced a feedback control on lipolysis, or to a deficiency in ATP, since energy is required for hormone-stimulated lipolysis. The finding by Angel et al. (30) of a reduction of adipocyte ATP by lipolytic agents including catecholamines may support this view.

There are few reports concerning the effects of CPZ on lipolysis in the adipose tissue. Torsti and Vapaatalo (10) and Hollister (9) reported an increase in plasma FFA levels after CPZ administration. Khan et al. (31) found that CPZ prevented the elevation of plasma FFA level in response to the stimuli of prolonged intermittent electric shocks. It has been also demonstrated that CPZ inhibited noradrenaline- or adrenaline-stimulated lipolysis in vitro (8).

In the experiments herein, the antilipolytic effect of CPZ on the spontaneous lipolysis was apparent only in the IBAT from cold-acclimated rats. Moreover, CPZ inhibited noradrenaline-stimulated lipolysis in IBAT and EWAT of cold-acclimated rats and the effect on IBAT was more evident than that on EWAT. After cold acclimation, the catecholamine concentration in brown adipose tissue increases (25), the density of catecholaminergic nerve terminals is also prominent (24, 25) and the release of noradrenaline from nerve terminals is enhanced (32). These are all signs of the elevated sympathetic activity. Therefore, it may be assumed that the effect of CPZ on IBAT from cold-acclimated rats is the result of a modification of action of noradrenaline.

Horwitz (33) reviewed studies on the biochemical thermogenesis of brown adipocyte and it was noted that such depends on noradrenaline, released from sympathetic nerves and that noradrenaline interacts primarily with the cell membrane, affecting at least 3 membrane properties; 1. activity of adenyl cyclase (34), 2. permeability of membrane (35), 3. activity of ouabain-sensitive Na⁺/K⁺ pump (36, 37). The enhanced activity of adenyl cyclase stimulates lipolysis and the free fatty acids being available as substrate in the mitochondrial energy production. Moreover, the increase in membrane permeability and concomitant increase in pump activity result in an elevation of the energy requirements of the cell. On the other hand, it was reported that CPZ inhibits the adenyl cyclase activity (38), membrane permeability (39, 40) and Na⁺,K⁺-ATPase activity (41, 42). Thus, antilipolytic effect of CPZ in brown adipose tissue from cold-acclimated rats is probably associated with a modification of the interaction of noradrenaline with the membrane of the adipocyte.

The inhibition of lipase activity by CPZ was observed only at the toxic concentration of $10^{-3}$ M and there was no significant effect seen with concentrations of $10^{-4}$ and $10^{-5}$ M. In contrast, $10^{-4}$ M CPZ clearly inhibited lipolysis in the IBAT of cold-acclimated rats.
The effective concentration of CPZ on the lipase activity was not in accordance with the antilipolytic concentration of CPZ in the IBAT from cold-acclimated rats. This discrepancy suggests that CPZ does not directly inhibit the lipase activity in the brown adipocytes.

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REFERENCES

1) SENAULT, C., HLUŠKO, M.-TH. AND PORTET, R.: Effects of diet and cold acclimation on lipid composition of rat interscapular brown adipose tissue. Ann. Nutr. Aliment. 29, 67–77 (1975)
2) PORTET, R., BEAUVALLET, M. AND SOULIER, M.: Variations of rat brown adipose tissue composition during cold acclimatization. Archs int. Physiol. Biochim. 84, 89–98 (1976)
3) DANIÉL, H. AND DERRY, D.M.: Criteria for differentiation of brown and white fat in the rat. Canad. J. Physiol. Pharmacol. 47, 941–945 (1969)
4) SMITH, R.E. AND HORNWITZ, B.A.: Brown fat and thermogenesis. Physiol. Rev. 49, 330–425 (1969)
5) HULL, D. AND SEGALL, M.M.: Sympathetic nervous control of brown adipose tissue and heat production in the new-born rabbit. J. Physiol. 181, 458–467 (1965)
6) KNIGHT, B.L. AND MYANT, N.B.: A comparison between the effects of cold exposure in vivo and of noradrenaline in vitro on the metabolism of the brown fat of new-born rabbits. Biochem. J. 119, 103–111 (1970)
7) FLATMARK, T. AND PEDERSEN, J.I.: Brown adipose tissue mitochondria. Biochim. biophys. Acta 416, 53–103 (1975)
8) FINGER, K.F., PAGE, J.G. AND FELLER, D.R.: Influence of various agonists and antagonists on the release of free fatty acids from adipose tissue in vitro. Biochem. Pharmacol. 15, 1023–1032 (1966)
9) DORIGO, P., GAION, R.M. AND FASSINA, G.: Lack of correlation between cyclic AMP synthesis and free fatty acid release in brown fat of cold-adapted rats. Biochem. Pharmacol. 23, 2877–2885 (1974)
10) BERTIN, R.: Glycerokinase activity and lipolysis regulation in brown adipose tissue of cold acclimated rats. Biochem. Pharmacol. 20, 1201–1211 (1971)
11) DORIGO, P., MARAGNO, I., BRETTA, A. AND FASSINA, G.: Reduced lipolytic response in vitro to catecholamines, ACTH and cyclic adenosine monophosphate in brown fat of cold-acclimated rats. Biochem. Pharmacol. 20, 1201–1211 (1971)
12) Novák, M.: Colorimetric ultramicro method for the determination of free fatty acids. J. Lipid Res. 6, 431–433 (1965)
13) RIZACK, M.A.: An epinephrine-sensitive lipolytic activity in adipose tissue. J. biol. Chem. 236, 657–662 (1961)
14) Ansell, G.B. AND BESSON, M.F.: A rapid and sensitive procedure for the combined assay of noradrenaline, dopamine, and serotonin in a single brain sample. Analyt. Biochem. 23, 196–206 (1968)
15) DORIGO, P., MARAGNO, I., BRESSA, A. AND FASSINA, G.: Reduced lipolytic response in vitro to catecholamines, ACTH and cyclic adenosine monophosphate in brown fat of cold-acclimated rats. Biochem. Pharmacol. 20, 1201–1211 (1971)
16) DORIGO, P., GAION, R.M. AND FASSINA, G.: Lack of correlation between cyclic AMP synthesis and free fatty acid release in brown fat of cold-adapted rats. Biochem. Pharmacol. 23, 2877–2885 (1974)
17) BERTIN, R.: Glycerokinase activity and lipolysis regulation in brown adipose tissue of cold acclimated rats. Biochemie 58, 431–434 (1976)
18) WIRSÉN, C.: Distribution of adrenergic nerve fibers in brown and white adipose tissue. Handbook of Physiol. V., Adipose Tissue, Edited by REYNOLDS, A.E. AND CHAILLÉ, G.F. JR., p. 197–199, Am. Physiol. Soc. (1965)
19) Stock, K. and Westermann, E.O.: Concentration of norepinephrine, serotonin, and histamine, and of amine-metabolizing enzymes in mammalian adipose tissue. *J. Lipid Res.* 4, 297–304 (1963)

20) Sidman, R.L., Perkins, M. and Weiner, N.: Noradrenaline and adrenaline content of adipose tissues. *Nature* 193, 36–37 (1962)

21) Cottle, M.K.W., Cottle, W.H. and Nash, C.W.: Adrenergic innervation of brown adipose tissue from the ground squirrel. *Canad. J. Physiol. Pharmacol.* 52, 70–73 (1974)

22) Derry, D.M., Schönbaur, E. and Steiner, G.: Two sympathetic nerve supplies to brown adipose tissue of the rat. *Canad. J. Physiol. Pharmacol.* 47, 57–63 (1969)

23) Derry, D.M. and Daniel, H.: Sympathetic nerve development in the brown adipose tissue of the rat. *Canad. J. Physiol. Pharmacol.* 48, 160–168 (1970)

24) Cottle, M.K.W. and Cottle, W.H.: Adrenergic fibers in brown fat of cold-acclimated rats. *J. Histochem. Cytochem.* 18, 116–119 (1970)

25) Huttunen, P., Vapaatalo, H. and Hirvonen, J.: Catecholamine content in the interscapular adipose tissue and adrenal gland of cold-acclimatized guinea-pigs. *Acta physiol. scand.* 93, 574–576 (1975)

26) Steiner, G. and Evans, S.: Sympathetic ganglia in brown adipose tissue: a new tool to study ganglionic stimulants. *Am. J. Physiol.* 222, 111–113 (1972)

27) Cantu, R.C. and Goodman, H.M.: Effects of denervation and fasting on white adipose tissue. *Am. J. Physiol.* 212, 207–212 (1967)

28) Joel, C.D.: Stimulation of metabolism of rat brown adipose tissue by addition of lipolytic hormones in vitro. *J. Biol. Chem.* 241, 814–821 (1966)

29) Kumon, A., Hara, T. and Takahashi, A.: Effects of catecholamines on the lipolysis of two kinds of fat cells from adult rabbit. *J. Lipid Res.* 17, 559–564 (1976)

30) Angel, A., Desai, K.S. and Halperin, M.L.: Reduction in adipocyte ATP by lipolytic agents: relation to intracellular free fatty acid accumulation. *J. Lipid Res.* 12, 203–213 (1971)

31) Khan, A.U., Forney, R.B. and Hughes, F.W.: Plasma free fatty acids in rats after shock as modified by centrally active drugs. *Archs int. Pharmacodyn. Thér.* 151, 466–474 (1964)

32) Cottle, W.H., Nash, C.W., Veress, A.T. and Ferguson, B.A.: Release of noradrenaline from brown fat of cold-acclimated rats. *Life Sci.* 6, 2267–2271 (1967)

33) Horwitz, B.A.: Physiological and biochemical characteristics of adrenergic receptors and pathways in brown adipocytes. *Temperature Regulation and Drug Action*, Edited by Lomax, P., Schönbaur, E. and Jacob, J., p. 150–158, Karger, Basel (1975)

34) Fain, J.N., Jacobs, M.D. and Clement-Cormier, Y.C.: Interrelationship of cyclic AMP, lipolysis, and respiration in brown fat cells. *Am. J. Physiol.* 224, 346–351 (1973)

35) Herd, P.A., Hammond, R.P. and Hamolsky, M.W.: Sodium pump activity during nor-epinephrine-stimulated respiration in brown adipocytes. *Am. J. Physiol.* 224, 1300–1304 (1973)

36) Herd, P.A., Horwitz, B.A. and Smith, R.E.M.: Norepinephrine-sensitive Na⁺/K⁺ ATPase activity in brown adipose tissue. *Experientia* 26, 825–826 (1970)

37) Horwitz, B.A.: Ouabain-sensitive component of brown fat thermogenesis. *Am. J. Physiol.* 224, 352–355 (1973)

38) Scatton, B., Bischoff, S., Dedek, J. and Korf, J.: Regional effects of neuroleptics on dopaminergic metabolism and dopaminergic-sensitive adenylate cyclase activity. *Europ. J. Pharmacol.* 44, 287–292 (1977)

39) Freeman, A.R. and Spirtes, M.A.: Effect of some phenothiazine derivatives on the hemolysis of red blood cells in vitro. *Biochem. Pharmacol.* 11, 161–173 (1962)

40) Spirtes, M.A. and Guth, P.S.: Effects of chlorpromazine on biological membranes—I. chlorpromazine-induced changes in liver mitochondria. *Biochem. Pharmacol.* 12, 37–46 (1963)

41) Judah, J.D. and Ahmed, K.: Inhibitors of transport and cation activated ATPases. *J. cell. comp. Physiol.* 64, 355–362 (1964)
42) Davis, P.W. and Brody, T.M.: Inhibition of Na⁺,K⁺-activated adenosine triphosphate activity in rat brain by substituted phenothiazines. *Biochem. Pharmacol.* **15**, 703–710 (1966)