Table 1. Effects of the oxamates **2** and **3** on UDP-glucose pyrophosphorylase specific activities in the *Echinococcus multilocularis* metacestodes and in the liver of infected Mongolian gerbil

	Livers activities	Metacestodes activities
Controls	198.67 ± 6.60	240 ± 29.44
Oxamate **2**	97.13 ± 5.39 ↘ (51%)	144.75 ± 7.37 ↘ (39.7%)
Oxamate **3**	70.58 ± 1.51 ↘ (64.5%)	51.44 ± 1.97 ↘ (78.6%)

UDP-glucose pyrophosphorylase activity is expressed as nmol·min^{-1}/mg proteins. Each value is the mean of three determinations ± SE.

Table 2. Repercussion of UDP-glucose pyrophosphorylase inactivation by oxamate **3** on glucose content in the *Echinococcus multilocularis* metacestodes and in the liver of infected Mongolian gerbil

	Livers content	Metacestodes content
Controls	2.77 ± 0.01	4.43 ± 0.11
Oxamate **3**	2.73 ± 0.01 ↘ (1%)	6.0 ± 0.01 ↗ (135%)

Glucose concentration is expressed as mmol/mg proteins. The results are means ± SE.

by oxamate **3** in liver than in metacestodes. The increased susceptibility of the metacestode enzyme could probably be due to the previously described differences in the kinetic and physical parameters of this enzyme compared to those from the liver of infected animals [4].

Table 3. Repercussion of UDP-glucose pyrophosphorylase inactivation by oxamate **3** on glycogen content in the *Echinococcus multilocularis* metacestodes and in the liver of infected Mongolian gerbil

	Livers content	Metacestodes content
Controls	0.014 ± 0.001	0.086 ± 0.001
Oxamate **3**	0 ↘ (100%)	0.236 ± 0.003 ↗ (270%)

Glycogen concentration is expressed as μmol/mg proteins. The results are means ± SE.

Acknowledgements—This work was supported by INSERM grants (Contract CRL INSERM CNAMTS 1984–1987).

**Laboratoire de Chimie Thérapeutique* — P. AUDIN*, E. SARCIRON†
†*Laboratoire de Parasitologie* — J. PARIS*
Faculté de Pharmacie — A. F. PETAVY†
8 Avenue Rockefeller
69373 Lyon Cédex 08, France

REFERENCES

1. A. I. Krotov, 4th International Congress of Parasitology, Warszawa, Section C, 19–26 August 1978.
2. T. Sakamoto, *Mem. Fac. Agr. Kagoshima Univ.* **15**, 115 (1979).
3. I. Delabre, C. Gabrion, F. Contat, A. F. Petavy and S. Deblock, *Int. J. Parasitol.* **3**, 773 (1987).
4. M. E. Sarciron, G. Azzar, F. Persat, A. F. Petavy and R. Got, *Molec. Biochem. Parasitol.* **23**, 25 (1987).
5. P. Audin, M. E. Sarciron, J. Paris and A. F. Petavy, *C.R. Acad Sci. Paris* **305**, Série III, 251 (1987).
6. A. Bensadoun and D. Weinstein, *Analyt. Biochem.* **70**, 241 (1976).
7. D. Kepler and K. Decker, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer) 2nd Edn, Vol. III, p. 1127. Verlag Chemie, Weinhein; Academic Press, New York (1974).

Biochemical Pharmacology, Vol. 37, No. 4, pp. 760–763, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Trifluoperazine does not inhibit the acute metabolic effects of insulin in rat adipocytes

(*Received* 4 *April* 1987; *accepted* 3 *August* 1987)

Calcium has been implicated in mediating some of the biochemical responses of insulin [1]. The effect of calcium in certain cases was attributed to its binding to calmodulin, a ubiquitous calcium binding protein [2]. Calmodulin has been demonstrated to activate a variety of enzymes, including several protein kinases [3–5], plasma membrane Ca^{2+}–Mg^{2+} ATPase [6] and cyclic nucleotide phosphodiesterase [2, 7]. A role for calmodulin in insulin action has been obtained directly from studies with calcium and calmodulin [8, 9], and also by inference from studies with "inhibitors" of calmodulin [10, 11]. The phenothiazines, specifically trifluoperazine, have been shown to inhibit calcium–calmodulin-activated processes [12]. There is not, however, a general consensus as to the role of calmodulin in the mechanism of insulin action.

Insulin is the major anabolic hormone regulating intermediary metabolism, yet the biochemical events coupling the insulin–receptor interaction with the modulation of intracellular processes remain to be fully elucidated [13]. Autophosphorylation of the insulin receptor is enhanced in response to insulin [14, 15] and in addition, "second messengers" are released into the intracellular milieu [16, 17]. Trifluoperazine can inhibit insulin receptor phosphorylation, but without altering the effect of insulin to stimulate glucose transport [18]. Since the effect of insulin to regulate glucose transport is dissociable from its

effect on intermediary metabolism [19, 20], this does not preclude the possibility that trifluoperazine could interfere with the mechanism by which insulin regulates metabolism. In fact, it has been suggested that trifluoperazine can modulate this latter aspect of insulin action [10, 11]. The effect of trifluoperazine could be mediated by inhibition of receptor autophosphorylation or insulin binding, or by inhibition of the insulin-signal transducing mechanism.

The present study was undertaken to investigate the possibility that trifluoperazine could inhibit the acute metabolic effects of insulin on isolated rat adipocytes. Three parameters were measured, one of which, glucose oxidation is a rapid event insensitive to the effect of "insulin messengers" [21]. The other two parameters, lipolysis and phospholipid methyltransferase are both exquisitely sensitive to insulin [9, 22, 23], and both are modulated by "insulin messengers" [20, 24–27].

Materials and methods

Materials. Male Sprague–Dawley rats were obtained from Hilltop Laboratories. Collagenase was from Worthington, bovine serum albumin (fraction V) was from US Biochemicals, and insulin was a gift from R. Chance of Eli Lilly. (1-^{14}C)-D-glucose and *S*-adenosyl-L-(methyl-^{3}H)-methionine were from New England Nuclear. All other chemicals were from standard sources.

Isolation and incubation of adipocytes. Adipocytes were isolated from the epidydimal pads of fed Sprague–Dawley rats, as previously described [23]. Adipocytes were isolated and incubated in a Krebs–Ringer bicarbonate buffer, pH 7.4, supplemented with 4% bovine serum albumin (BSA). Isolated fat cells were incubated in separate polyethylene vials, shaking at 37°.

Glucose oxidation was assayed as described in [28]. Briefly, adipocytes (4×10^4 cells/ml) were incubated for 1 hr at 37°, in a final volume of 2 ml containing (1-^{14}C)D-glucose (0.2 mM) and insulin, in the absence or presence of trifluoperazine. Glucose oxidation was measured as the rate of ^{14}C–CO_2 released.

Lipolysis was measured as the accumulation of glycerol release, as in [19, 29]. Adipocytes (1×10^6 cells/ml) were preincubated for 30 min at 37° as 1 ml aliquots. Isoproterenol, insulin and trifluoperazine were subsequently added for an additional 15 min, after which the cells were separated from the bathing medium by centrifugation. Glycerol released into the medium was assayed fluorimetrically as in [29].

Phospholipid methyltransferase activity was measured in homogenates of adipocytes prepared following hormone treatment, as in [27]. Adipocytes (1×10^6 cells/ml) were preincubated for 30 min at 37° as 1 ml aliquots. Test agents were added for an additional 20 min, after which the cells were separated from the bathing medium by centrifugation. Adipocytes were lysed with the addition of cold 10 mM Tris–HCl, pH 8.5 containing 10 mM KF, 2 mM EGTA and 2 mM EDTA, and a fat-free infranatant prepared by centrifugation for 1 min at 10,000 *g*.

Results

Effect of trifluoperazine on insulin action

Glucose oxidation. Rat adipocytes (4×10^4 cells/ml) were incubated with increasing concentrations of insulin in the absence and presence of trifluoperazine for 1 hr (Fig. 1). Glucose oxidation was measured in the presence of a low concentration of glucose (0.2 mM), such that glucose transport was rate-limiting. Under these conditions, the effect of insulin on glucose oxidation indirectly reflected the effect on glucose transport [21, 30]. Treatment of adipocytes with insulin caused a concentration-dependent increase in glucose oxidation. Insulin (100 μU/ml) caused a 5-fold increase in glucose oxidation with a half-maximal effect at 25 μU/ml. Trifluoperazine at concentrations of 12, 50 and 100 μM had no effect on either the EC_{50} for insulin or the maximum response to insulin (Fig. 1). There was a slight inhibitory effect of 100 μM trifluoperazine on insulin-stimulated glucose oxidation, but the physiological significance of this effect is questionable due to the high concentration of trifluoperazine used.

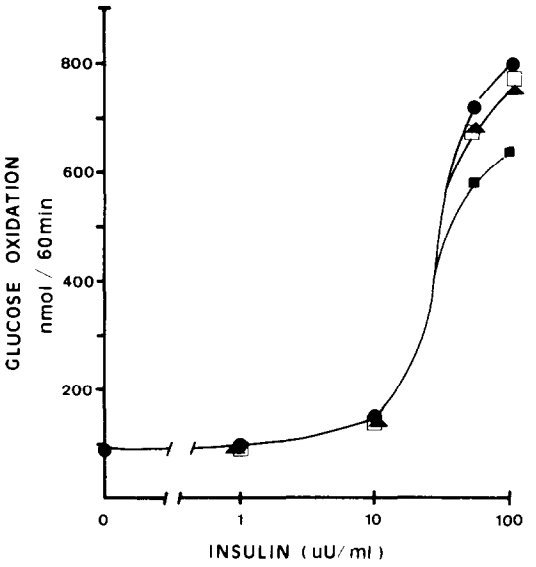

Fig. 1. Effect of trifluoperazine on insulin-stimulated glucose oxidation. Rat adipocytes were incubated with increasing concentrations of insulin (0, 1, 10, 50 or 100 μU/ml) in the absence (●) or presence of trifluoperazine 12 μM (□), 50 μM (▲) or 100 μM (■) for 1 hr. Glucose oxidation was measured as the release of $^{14}CO_2$. Results are the average of 3 individual experiments performed in triplicate; standard errors were less than 10%.

Antilipolysis. Treatment of rat adipocytes (1×10^6 cells/ml) for 15 min with 100 nM isoproterenol caused a 12-fold increase in glycerol release (Fig. 2). Insulin inhibited the lipolytic effect of isoproterenol in a concentration-dependent manner, with an IC_{50} of 25 μU/ml. The addition of trifluoperazine at 12, 50 and 100 μM had no effect on the antilipolytic response of insulin. Trifluoperazine in the absence of insulin, had a slight inhibitory effect of its own on isoproterenol-stimulated glycerol release.

Phospholipid methyltransferase. Treatment of adipocytes (1×10^6 cells/ml) for 20 min with 10 mM isoproterenol caused a 3-fold increase in phospholipid methyltransferase activity, and this was inhibited in a concentration-dependent manner by insulin (Fig. 3). The IC_{50} for insulin was 10 μU/ml, and insulin at 100 μU/ml caused an 80% inhibition of isoproterenol-stimulated phospholipid methyltransferase activity. Incubation of adipocytes with isoproterenol and insulin in the presence of increasing concentrations of trifluoperazine had no effect on insulin action. There was a slight inhibition (10 ± 2%) by trifluoperazine alone on isoproterenol-stimulated phospholipid methyltransferase activity. This effect of trifluoperazine was not observed on basal phospholipid methyltransferase activity, and it was not concentration dependent (Fig. 3).

Phospholipid methyltransferase is activated by phosphorylation mediated by both cAMP-independent and -independent kinases [19, 23, 31–33]. Incubation of adipocytes with oxytocin also causes a stimulation of phospholipid methyltransferase (Table 1) via a cAMP-independent mechanism [19]. Oxytocin is albeit a weak stimulus for phospholipid methyltransferase, but the effect of oxytocin is cAMP-independent and thought to be a calcium-

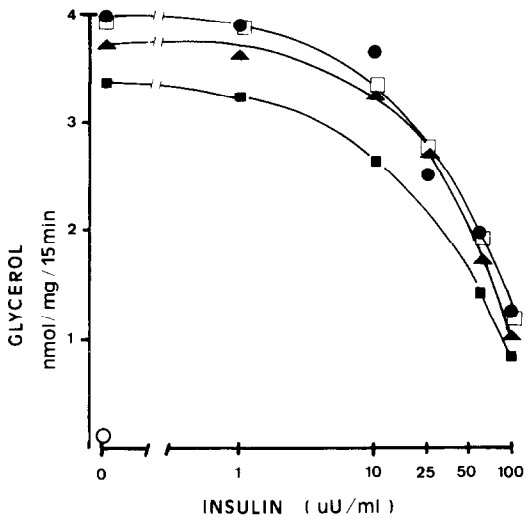

Fig. 2. Effect of trifluoperazine on the antilipolytic effect of insulin. Rat adipocytes were incubated with 100 nM isoproterenol and increasing concentrations of insulin (1, 10, 25, 50 or 100 μU/ml) in the absence (●) or presence of trifluoperazine 12 μM (□), 50 μM (▲), or 100 μM (■) for 15 min. Lipolysis was measured as the release of glycerol. Results are the average of 3 individual experiments performed in triplicate; standard errors were less than 5%.

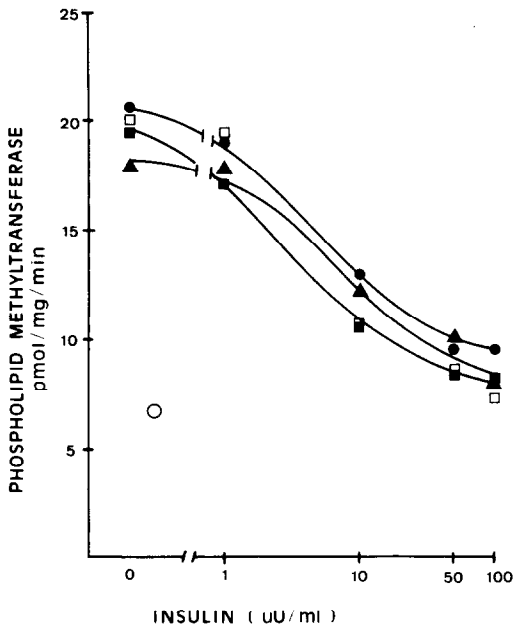

Fig. 3. Effect of trifluoperazine on the inhibitory effect of insulin on isoproterenol-stimulated phospholipid methyltransferase. Rat adipocytes were incubated with 10 nM isoproterenol and increasing concentrations of insulin (1, 10, 50 or 100 μU/ml) in the absence (●) or presence of trifluoperazine 12 μM (□), 50 μM (▲) or 100 μM (■) for 20 min. Phospholipid methyltransferase was measured as the incorporation of (methyl-^{3}H) into phospholipids. Control activity (○) was 6.6 ± 0.5 pmol/mg/min. Results are the average of 3 individual experiments performed in triplicate; standard errors were less than 10%.

mediated event [19]. Hence, trifluoperazine effectively abolished the stimulation of phospholipid methyl-transferase by oxytocin. These data suggest that although trifluoperazine does not interfere in the mechanism of insulin action on phospholipid methyltransferase, it can interfere in the activation of phospholipid methyltransferase in response to agents, like oxytocin, which are thought to act through calcium.

Discussion

The present study was undertaken to test the hypothesis that trifluoperazine abolishes an obligatory biochemical event in insulin-response coupling. Trifluoperazine at concentrations as high as 100 μM, did not interfere with the effect of insulin to stimulate glucose oxidation, or to inhibit isoproterenol-stimulated lipolysis or phospholipid methyltransferase. These data are in agreement with those of [10, 11, 18], who demonstrated that the binding of insulin in adipocytes, and the biochemical events leading to insulin-mediated glucose transport and antilipolysis are not affected by trifluoperazine. They contrast, however, with those of Ref. 11, who found that 70 μM trifluoperazine caused a 50% inhibition of glucose transport. Furthermore, although these data are similar to those reported by [10], they are not in agreement with the conclusions from these studies. Begum *et al.* [10] concluded that calmodulin was involved in the mechanism by which insulin stimulated pyruvate dehydrogenase, purportedly in the release or action of an "insulin second messenger". It was previously demonstrated that both lipolysis and phospholipid methyltransferase are modulated by a phospho-oligosaccharide [26, 27] that appears to function as an insulin second messenger. This same compound mimics the effect of insulin to inhibit pyruvate dehydrogenase [34] without altering glucose transport [26]. It appears from these studies that the mechanisms by which insulin regulates antilipolysis, phospholipid methyltransferase and pyruvate dehydro-

Table 1. Inhibition of oxytocin-stimulated phospholipid methyltransferase by trifluoperazine

	Phospholipid methyltransferase activity (pmol/mg/min)	% Inhibition
Control	5.9 ± 0.10	
Oxytocin 1 μM	8.5 ± 0.20	
+TFP 1 μM	7.6 ± 0.16	35%
+TFP 10 μM	7.7 ± 0.41	35%
+TFP 100 μM	5.3 ± 0.04	100%

Rat adipocytes were incubated with oxytocin in the absence and presence of increasing concentrations of trifluoperazine for 20 min. Results are the average ±SEM of a representative experiment performed in triplicate.

genase are similar. Despite the evidence that calmodulin is involved in insulin–receptor autophosphorylation [18], and in the ATP-dependent Ca^{2+} transport of adipocyte plasma membranes [6, 8, 9], there does not appear to be an obligatory step in insulin signalling that is obliterated when adipocytes are incubated with trifluoperazine.

Trifluoperazine does inhibit the effect of oxytocin to stimulate phospholipid methyltransferase, suggesting that calmodulin may be involved in mediating the effect of oxytocin on this enzyme.

The data from this study suggest a reevaluation of the role of calmodulin in insulin action as determined by studies with trifluoperazine [10, 11], the most potent inhibitor of calmodulin [12].

Acknowledgements—The authors wish to thank Judy Smith for her technical assistance, and Drs Jose Mato and Leonard Jarett for their discussions. This work was supported by grants from the American Diabetes Association and the American Heart Society.

University of Pennsylvania
School of Medicine
Department of Pathology and
Laboratory Medicine
Philadelphia, PA 19104, U.S.A.

KATHLEEN L. KELLY*
DINA DICENZO

REFERENCES

1. H. Rasmussen and D. Waisman, in *Biochemical Actions of Hormones*, Vol. 8 (Ed. G. Litwack), p. 2. Academic Press, New York (1981).
2. W. Y. Cheung, *J. biol. Chem.* **246**, 2859 (1971).
3. W. Y. Cheung, *Science* **207**, 19 (1980).
4. W. Y. Cheung, in *Calcium and Cell Function*, Vol. 1 (Ed. W. Y. Cheung), p. 2. Academic Press, New York (1980).
5. E. G. Krebs and J. A. Beavo, *Ann. Rev. Biochem.* **48**, 923 (1979).
6. J. M. McDonald, K.-M. Chan, R. R. Glewert, R. A. Mooney and H. A. Pershadsingh, *Ann. N.Y. Acad. Sci.* **402**, 381 (1982).
7. S. Kakiuchi and R. Yamazak, *Biochem. biophys. Res. Commun.* **41**, 1104 (1970).
8. R. G. Goewert, N. B. Klaven and J. M. McDonald, *J. biol. Chem.* **258**, 9995 (1983).
9. M. Landt and J. M. McDonald, *Biochem. biophys. Res. Commun.* **93**, 881 (1980).
10. N. Begum, H. M. Tepperman and J. Tepperman, *Endocrinology* **118**, 287 (1986).
11. Y. Shechter, *Proc. natn. Acad. Sci. U.S.A.* **81**, 327 (1984).
12. R. Levin and B. Weiss, *Molec. Pharmac.* **13**, 690 (1977).
13. M. Czech, *Am. J. Med.* **70**, 142 (1981).
14. M. Kasuga, Y. Zick, D. L. Blith, F. A. Karlsson, H. U. Haring and C. R. Kahn, *J. biol. Chem.* **257**, 9891 (1982).
15. L. M. Petruzellii, S. Ganguly, C. J. Smith, M. H. Cobb, S. Rubin and O. Rosen, *Proc. natn. Acad. Sci. U.S.A.* **79**, 6792 (1982).
16. J. Larner, L. C. Huang, G. Brooker, G. Murad and T. B. Miller, *Fedn Proc. Fedn Am. Socs exp. Biol.* **233**, 261 (1974).
17. J. Seals and L. Jarett, *Science* **206**, 1407 (1979).
18. W. E. Plehwe, P. F. Williams, I. D. Caterson, L. C. Harrison and J. R. Turtle, *Biochem. J.* **214**, 361 (1983).
19. C. Villar-Palasi and J. Larner, *Archs Biochem. Biophys.* **94**, 436 (1961).
20. T. D. Gelehrter, P. D. Shreve and V. M. Dilworth, *J. biol. Chem.* **239**, 375 (1964).
21. L. Jarett, E. H.-A. Wong, S. L. Macaulay and J. A. Smith, *Science* **227**, 533 (1985).
22. R. L. Jungas and E. G. Ball, *Biochemistry* **2**, 383 (1963).
23. K. L. Kelly, E. H.-A. Wong and L. Jarett, *J. biol. Chem.* **260**, 3640 (1985).
24. K. L. Kelly and E. H.-A. Wong, *Endocrinology*, **120**, 2421 (1987).
25. S. R. Zhang, W. H.-Shi and R. J. Ho, *J. biol. chem.* **258**, 6471 (1983).
26. K. L. Kelly, I. Merida, J. M. Mato and L. Jarett, *Proc. natn. Acad. Sci. U.S.A.* **84**, 6404 (1987).
27. K. L. Kelly, J. M. Mato and L. Jarett, *FEBS Lett.* **209**, 238 (1986).
28. L. Jarett and R. Smith, *J. biol. Chem.* **249**, 7024 (1974).
29. O. Wieland, *Biochem. Z.* **329**, 3 (1957).
30. O. B. Crofford and A. E. Renold, *J. biol. Chem.* **240**, 14 (1965).
31. I. Varela, I. Merida, M. Villalba and J. M. Mato, *Biochem. biophys. Res. Commun.* **122**, 1065 (1984).
32. I. Merida, I. Varela, J. F. Alvarez, C. Cabrero and J. M. Mato, *FEBS Lett.* **196**, 274 (1986).
33. K. L. Kelly, *Biochem. J.* **241**, 917 (1987).
34. A. R. Saltiel, *Endocrinology* **120**, 967 (1987).

* To whom reprint requests should be sent.

Biochemical Pharmacology, Vol. 37, No. 4, pp. 763–766, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Substituted phenylpiperidines and phenylpyridines as reversible selective inhibitors of monoamine oxidase type A in rodent brain and liver

(*Received* 8 *June* 1987; *accepted* 28 *July* 1987)

Clinically effective antidepressant monoamine oxidase (MAO) inhibitors share the property of inhibiting MAO-A, which is characteristic of monoaminergic nerve terminals [1]. These agents as well as the newer, selective propargylamine inhibitors of MAO-A (clorgyline) and MAO-B ([-]deprenyl) are irreversible agents with prolonged actions [1]. The older, clinically effective non-selective MAO inhibitors and the selective anti-MAO-A agents also potentiate the pressor effects of indirect sympathomimetics such as tyramine, sometimes dangerously, by complex and incompletely defined mechanisms [1, 2]. Several new reversible short-acting inhibitors of MAO-A (in addition to the older harmala alkaloids) are currently under investigation (cimoxatone, moclobemide, and others) [2]. At least one of these, the benzofuranyl piperidine derivative brofaromine (CGP-11-305-A) [3], may be a clinically effective antidepressant and also have less risk of inducing hypertension than the older drugs [2]. Further leads to additional novel, selective and reversible MAO inhibitors may arise from recent studies of the pharmacologic effects of certain neurotoxic phenylpyridines [4].

Inhibitors of MAO, especially of type B, reportedly