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EFFECT OF PYRAZOLE ON LIPOLYSIS *IN VITRO*

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Abstract—The effects of pyrazole on the basal release as well as on the theophylline stimulated release of glycerol and FFA from rat epididymal adipose tissue were studied *in vitro*, and compared to those of 5-methyl-pyrazole-3-carboxylic acid (MPC).

Unlike MPC, pyrazole did not significantly affect the basal release of glycerol, but reduced only the basal release of FFA. It decreased the theophylline stimulated glycerol and FFA release, its action being, however, much weaker than that of MPC. Pyrazole within adipose tissue may have two different sites of action; first, and like MPC, a stimulation of phosphodiesterase, secondly, and opposite to MPC, a stimulation of the re-esterification process.

PYRAZOLE, a potent competitive inhibitor of alcohol dehydrogenase (ADH),[1] is frequently used for studying the metabolic disorders induced by ethanol. Because of its controversed effect on the ethanol-induced fatty liver,[2–4] our experiments in this field[5] has led us to elucidate whether pyrazole influences or not lipolysis. Referring to the antilipolytic action of many pyrazole derivatives,[6,7] Lieber recently suggested that pyrazole might inhibit peripheral fatty mobilization but added that no study of this problem has been done to his knowledge.[8] We have tested this hypothesis by studying, on rat epididymal adipose tissue *in vitro*, the effect of pyrazole, both on basal and theophylline stimulated lipolysis. It was considered also to be of interest to compare these results with those obtained in the same conditions with 5-methyl-pyrazole-3-carboxylic acid (MPC), a very potent antilipolytic agent.

MATERIAL AND METHODS

Incubation of adipose tissue in vitro

Male Wistar rats weighing 200 ± 20 g and fasted for 18 hr, were anesthesized with diethylether and decapited. For each animal both distal portions of the epididymal fat pads were excised, cut into pieces and incubated in duplicate in such a way that each flask contained two pieces from each epididymal fat pad (total tissue weight per flask being 180–200 mg). The incubation medium (5 ml) consisted of 5% (w/v) bovine albumin (fraction v, Miles Pentex), $5 \cdot 10^{-3}$ M glucose, in Krebs–Ringer-bicarbonate buffer, pH 7·4.[9] In order to determine the time during which the action persists, the incubations were performed for one or three hours at 37° with gentle shaking. Air served as gas phase. Additions to the medium were done at zero time. Pyrazole (Fluka) and MPC (Upjohn) were added as well at different concentrations. Theophylline (Merck) was added to the incubation medium at the concentration 5.10^{-3} M. At the end of each incubation, the pH of the medium was still 7·4.

Analytical methods

Lipolysis in adipose tissue was determined by the rate of glycerol and free fatty acids (FFA) release in the medium. Aliquots, taken off from the medium after incubation, were immediately extracted for the determination of FFA according to Dole.[10] Glycerol was assayed by the coupled enzymatic system of Eggstein.[11] Productions of FFA and glycerol are expressed respectively as microequivalents and micromoles liberated to the medium per gram adipose tissue in 1 or 3 hr.

The statistical evaluation of the results was carried out by the Student's *t*-test.

RESULTS

Effect on basal release from adipose tissue

The results on the effect of addition at different concentrations of either pyrazole or MPC on the release of glycerol and FFA after one hr incubation are given in Table 1. As can be seen, the addition of 10^{-4} and 10^{-3} M pyrazole does not affect the basal release of glycerol, while the same MPC concentrations reduce significantly the production of glycerol. Pyrazole and MPC show, however, the same lowering activity on FFA release. The response is dose–dependent in the case of both agents.

After 3 hr incubation, on the other hand, 10^{-4} M pyrazole and MPC do not have any significant effect on basal lipolysis (Table 2). At 10^{-3} M, however, lipolytic activity is slightly decreased by both compounds. The depression is maximal at 10^{-2} M, the rate of inhibition being less than after 1 hr incubation.

Effect on the theophylline stimulated release from adipose tissue

The same type of experiments has been performed with addition of theophylline (5.10^{-3} M) to the medium in order to determine whether pyrazole is effective in reducing the *in vitro* stimulated lipolysis. Table 3 shows that after 1 hr the lipolytic rate is more markedly decreased by pyrazole than when using basal conditions. This dose–dependent effect influences release of both glycerol and FFA. Maximal inhibition is obtained at 10^{-2} M concentration, the amounts of glycerol and FFA liberated from the tissue being respectively $67{\cdot}2 \pm 9$ and $55{\cdot}6 \pm 6$ per cent of the control values. In the same conditions, the antilipolytic effect of MPC is greater, since at 10^{-2} M, glycerol and FFA productions represent less than a third of the control values. At 10^{-4} M, MPC is still effective, (the glycerol production being depressed by 50 per cent), whereas pyrazole has no appreciable effect. Therefore, pyrazole 10^{-2} M shows approximately the same antilipolytic effect on theophylline stimulated lipolysis than MPC 10^{-4} M.

After three hr incubation (Table 4), the addition of pyrazole has no significant influence on the theophylline stimulated glycerol release, whereas the FFA production is still decreased. With 10^{-2} M pyrazole it reaches 63 per cent of the control values. The addition of MPC provides different results: the rate of inhibition previously found after 1 hr persists after 3 hr and influences to the same extent the production of both glycerol and FFA.

For all experiments variations of the esterification index ($3 \times$ glycerol release—FFA release) are presented in Table 5.

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TABLE 1. EFFECTS OF PYRAZOLE AND MPC ON 1 hr SPONTANEOUS GLYCEROL AND FFA RELEASE OF ADIPOSE TISSUE FROM 18 hr FASTED RATS

Drugs in the medium M conc.	Glycerol in the medium (μM/g/60 min)	FFA in the medium (μEquiv./g/60 min)	Drugs in the medium M conc.	Glycerol in the medium (μM/g/60 min)	FFA in the medium (μEquiv./g/60 min)
—	1·60 ± 0·18*	5·43 ± 0·44*		1·64 ± 0·14‡	5·62 ± 0·30‡
Pyrazole 10^{-4}	1·44 ± 0·13*	4·27 ± 0·46*	MPC 10^{-4}	1·28 ± 0·10‡	4·27 ± 0·47‡
	(0·1 < P < 0·2)	(0·001 < P < 0·01)		(0·001 < P < 0·01)	(0·001 < P < 0·01)
—	1·58 ± 0·21*	3·86 ± 0·22*		1·59 ± 0·16‡	4·45 ± 0·05‡
Pyrazole 10^{-3}	1·41 ± 0·20*	2·36 ± 0·19*	MPC 10^{-3}	1·06 ± 0·07‡	2·99 ± 0·33‡
	(0·2 < P < 0·3)	(P < 0·001)		(P < 0·001)	(P < 0·001)
—	1·62 ± 0·15*	4·06 ± 0·31*		1·60 ± 0·12†	4·49 ± 0·08†
Pyrazole 10^{-2}	1·32 ± 0·15*	2·15 ± 0·34*	MPC 10^{-2}	0·88 ± 0·19†	2·16 ± 0·16†
	(0·01 < P < 0·02)	(P < 0·001)		(P < 0·001)	(P < 0·001)

Minces of epididymal fat pads (200 mg) were incubated in 5 ml Krebs–Ringer-Bicarbonate buffer (pH 7·4) containing 5% bovine albumin and 10^{-3} M glucose, at 37° for 1 hr, in a metabolic shaker. Each value represents the mean ± S.E. of (*) ten assays, of (†) six assays and of (‡) four assays.

TABLE 2. EFFECTS OF PYRAZOLE AND MPC ON 3 hr SPONTANEOUS GLYCEROL AND FFA RELEASE OF ADIPOSE TISSUE FROM 18 hr FASTED RATS

Drugs in the medium M conc.	Glycerol in the medium (μM/g/180 min)	FFA in the medium (μEquiv./g/180 min)	Drugs in the medium M conc.	Glycerol in the medium (μM/g/180 min)	FFA in the medium (μEquiv./g/180 min)
—	4·71 ± 0·23*	5·49 ± 0·22*		4·82 ± 0·30†	2·75 ± 0·44†
Pyrazole 10^{-4}	4·45 ± 0·67*	5·51 ± 0·51*	MPC 10^{-4}	4·54 ± 0·46†	2·47 ± 0·38†
	(P > 0·4)	(P > 0·9)		(P > 0·3)	(P > 0·3)
—	4·70 ± 0·18*	4·13 ± 0·49*		4·64 ± 0·42†	3·61 ± 0·30†
Pyrazole 10^{-3}	4·32 ± 0·20*	3·51 ± 0·11*	MPC 10^{-3}	3·80 ± 0·53†	2·99 ± 0·15†
	(0·01 < P < 0·02)	(0·02 < P < 0·05)		(0·02 < P < 0·05)	(0·001 < P < 0·01)
—	5·40 ± 0·47*	6·25 ± 0·35*		5·00 ± 0·19*	5·39 ± 0·20*
Pyrazole 10^{-2}	4·38 ± 0·29*	4·43 ± 0·36*	MPC 10^{-2}	3·60 ± 0·21*	3·66 ± 0·15*
	(0·001 < P < 0·01)	(P < 0·001)		(P < 0·001)	(P < 0·001)

Minces of epididymal fat pads were incubated during 3 hr with experimental conditions as in Table 1. Each value represents the mean ± S.E. of (*) five assays and of (†) four assays.

TABLE 3. EFFECTS OF PYRAZOLE AND MPC ON 1 hr THEOPHYLLINE STIMULATED GLYCEROL AND FFA RELEASE OF ADIPOSE TISSUE FROM 18 hr FASTED RATS

Drugs in the medium M conc.	Glycerol in the medium (μM/g/60 min)	FFA in the medium (μEquiv./g/60 min)	Drugs in the medium M conc.	Glycerol in the medium (μM/g/60 min)	FFA in the medium (μEquiv./g/60 min)
—	9·02 ± 0·42‡	21·99 ± 0·94‡		8·16 ± 0·86§	22·65 ± 2·75§
Pyrazole 10^{-4}	9·14 ± 0·30‡	21·34 ± 0·98‡	MPC 10^{-4}	4·12 ± 0·53§	16·61 ± 2·04§
	(P > 0·9)	(P > 0·9)		(P < 0·001)	(P < 0·001)
—	10·28 ± 0·97*	26·46 ± 1·89*		7·85 ± 1·23§	21·65 ± 1·31§
Pyrazole 10^{-3}	8·39 ± 0·98*	20·21 ± 2·64*	MPC 10^{-3}	3·46 ± 0·59§	11·02 ± 1·44§
	(0·01 < P < 0·02)	(0·001 < P < 0·01)		(P < 0·001)	(P < 0·001)
—	10·13 ± 0·84†	26·76 ± 2·15†		9·95 ± 0·93§	22·83 ± 2·05§
Pyrazole 10^{-2}	6·81 ± 0·91†	14·88 ± 1·58†	MPC 10^{-2}	3·78 ± 0·55§	8·14 ± 0·68§
	(0·001 < P < 0·01)	(P < 0·001)		(P < 0·001)	(P < 0·001)

Experimental conditions as in Table 1. Theophylline (5.10^{-3} M) was added to the medium. Each value represents the mean ± S.E. of (*) ten assays, of (†) eight assays, of (‡) five assays and of (§) four assays.

TABLE 4. EFFECTS OF PYRAZOLE AND MPC ON 3 hr THEOPHYLLINE STIMULATED GLYCEROL AND FFA RELEASE OF ADIPOSE TISSUE FROM 18 hr FASTED RATS

Drugs in the medium M conc.	Glycerol in the medium (μM/g/180 min)	FFA in the medium (μEquiv./g/180 min)	Drugs in the medium M conc.	Glycerol in the medium (μM/g/180 min)	FFA in the medium (μEquiv./g/180 min)
—	17·99 ± 0·60*	36·46 ± 1·34*		19·69 ± 1·72†	40·01 ± 7·70†
Pyrazole 10^{-4}	18·04 ± 0·63*	33·55 ± 1·82*	MPC 10^{-4}	12·85 ± 1·10†	22·24 ± 2·88†
	(P > 0·9)	(P > 0·5)		(P < 0·001)	(0·001 < P < 0·01)
—	17·54 ± 1·72*	37·38 ± 3·23*		16·07 ± 2·57†	26·83 ± 6·63†
Pyrazole 10^{-3}	17·29 ± 1·76*	26·68 ± 3·10*	MPC 10^{-3}	8·90 ± 1·02†	13·45 ± 1·97†
	(P > 0·8)	(0·001 < P < 0·01)		(0·001 < P < 0·01)	(0·001 < P < 0·01)
—	18·43 ± 0·87*	36·17 ± 3·58*		18·40 ± 1·04†	35·15 ± 3·31†
Pyrazole 10^{-2}	17·09 ± 1·97*	22·92 ± 0·52*	MPC 10^{-2}	6·44 ± 0·70†	11·26 ± 1·49†
	(0·2 < P < 0·3)	(P < 0·001)		(P < 0·001)	(P < 0·001)

Experimental conditions as in Table 2. Theophylline (5.10^{-3} M) was added to the medium. Each value represents the mean ± S.E. of (*) five assays and of (†) four assays.

TABLE 5. EFFECTS OF PYRAZOLE AND MPC ON THE ESTERIFICATION INDEX OF ADIPOSE TISSUE FROM 18 hr FASTED RATS

Drugs in the medium M conc.	Esterification index (3× glycerol release—FFA release)			
	Basal lipolysis		Theophylline stimulated lipolysis	
	1 hr (A)	3 hr (B)	1 hr (C)	3 hr (D)
—	0·63 ± 0·55*	+ 8·61 ± 1·25‡	+ 5·08 ± 0·82‡	+ 17·51 ± 0·46‡
Pyrazole 10^{-4}	+ 0·05 ± 0·52*	+ 7·84 ± 1·95‡	+ 6·05 ± 0·86‡	+ 20·57 ± 1·89‡
	(0·1 < P < 0·2)	(P > 0·5)	(0·1 < P < 0·2)	(0·01 < P < 0·02)
—	− 0·70 ± 0·34§	+ 11·71 ± 0·97§	+ 1·85 ± 1·54§	+ 19·06 ± 2·91§
MPC 10^{-4}	− 0·43 ± 0·61§	+ 11·15 ± 1·14§	− 4·25 ± 2·00§	+ 16·29 ± 1·69§
	(P > 0·4)	(P > 0·4)	(0·001 < P < 0·01)	(0·1 < P < 0·2)
—	+ 0·88 ± 0·66*	+ 9·99 ± 0·64‡	+ 4·38 ± 3·28*	+ 15·29 ± 3·20‡
Pyrazole 10^{-3}	+ 1·87 ± 0·70*	+ 9·45 ± 0·57‡	+ 4·96 ± 2·34*	+ 25·19 ± 5·15‡
	(0·02 < P < 0·05)	(0·2 < P < 0·3)	(P > 0·7)	(0·01 < P < 0·02)
—	+ 0·32 ± 0·44§	+ 10·30 ± 1·13§	+ 1·90 ± 2·73§	+ 21·38 ± 2·10§
MPC 10^{-3}	+ 0·19 ± 0·31§	+ 8·41 ± 1·10§	− 0·64 ± 2·13§	+ 13·25 ± 4·12 §
	(P > 0·3)	(P = 0·05)	(0·1 < P < 0·2)	(0·01 < P < 0·02)
—	+ 0·80 ± 0·42*	+ 9·95 ± 1·44‡	+ 3·63 ± 2·47**	+ 19·12 ± 3·00‡
Pyrazole 10^{-2}	+ 1·81 ± 0·63*	+ 8·71 ± 0·79‡	+ 5·55 ± 3·47**	+ 28·35 ± 3·40‡
	(0·01 < P < 0·02)	(0·1 < P < 0·2)	(P > 0·3)	(0·001 < P < 0·01)
—	+ 0·33 ± 0·31†	+ 9·60 ± 0·53‡	+ 7·01 ± 1·17§	+ 20·05 ± 1·38§
MPC 10^{-2}	+ 0·47 ± 0·55†	+ 7·14 ± 0·64‡	+ 3·20 ± 1·29§	+ 8·06 ± 1·60§
	(P > 0·3)	(P < 0·001)	(0·001 < P < 0·01)	(P < 0·001)

Experimental conditions as (A) in Table 1, as (B) in Table 2, as (C) in Table 3, as (D) in Table 4. Each value represents the mean ± S.E. of (*) ten assays, of (**) eight assays, of (†) six assays of (‡) five assays and of (§) four assays.

DISCUSSION

The breakdown of triglycerides is mediated within adipose tissue through the hormone sensitive lipase activity,[12] and yields FFA and glycerol which eventually reach the blood stream. This process is reversible to a considerable degree for the released FFA, which can be either re-esterified to form triglycerides or oxidised to yield acetyl CoA. On the contrary glycerol once liberated, does not appear to be re-esterified within white adipose cells.[13] Glycerol is therefore, considered to be the final breakdown product during lipolysis and is classically accepted to be a better indicator of the lipolytic process than FFA. It is interesting to determine, in this connection, the tissue capacity of FFA re-esterification[14] by measuring the *esterification index*.

Among the numerous antilipolytic agents available in recent years, the pyrazole derivatives group is one of the most potent, inhibiting both basal and stimulated lipolysis in adipose tissue.[6,7,15,16] Our results show that MPC decreases both FFA and glycerol release. Pyrazole, opposite to MPC, decreases in basal conditions only the FFA release (Fig. 1). This finding suggests that pyrazole may enhance either the oxidation or the re-esterification of the FFA released by spontaneous lipolysis. In the conditions of incubation used (with addition of glucose, substrate which is known to inhibit fatty acid oxidation),[17] it can be assumed that pyrazole enhances the re-esterification process.

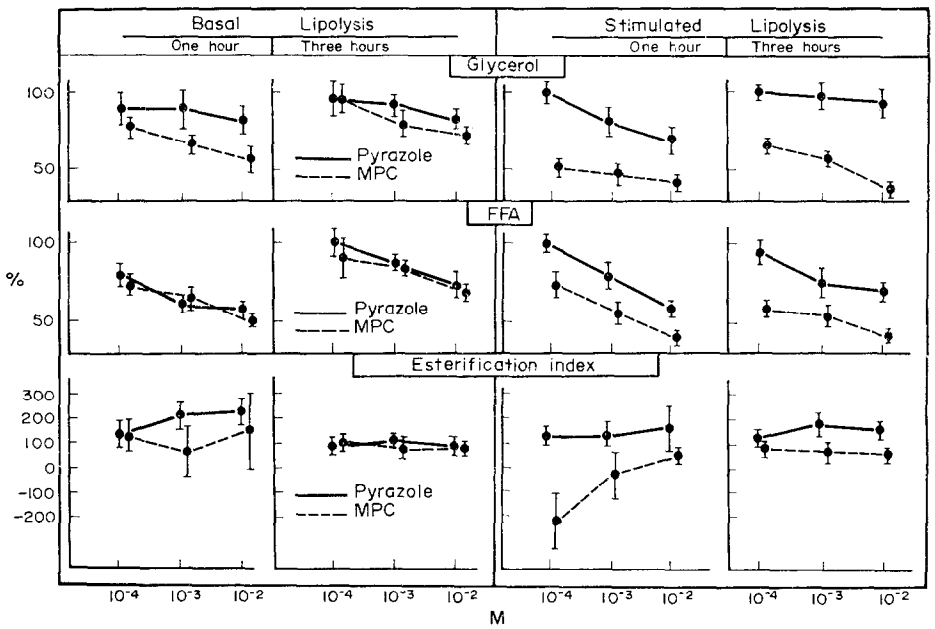

FIG. 1. Effects of pyrazole and MPC on spontaneous and stimulated release of glycerol and FFA from, and on calculated *esterification index* of, epididymal adipose tissue from 18 hr starved rats incubated *in vitro*. Each bar represents the mean ± S.E. of the percentage of the control values calculated from the results included in Tables 1–5.

Opposite to the results on basal lipolysis, pyrazole has a clear cut antilipolytic effect on the theophylline stimulated lipolysis. As this effect is analogous to the one observed with MPC, it seems that inhibition of enhanced lipolysis by pyrazole is mediated through the same mechanism[18] previously described for other pyrazole-derivatives,[6] especially MPC,[19] and for nicotinic acid.[20] These compounds do not directly inhibit the conversion of inactive to active hormone-sensitive lipase through cyclic adenosine 3′,5′-monophosphate, but affect the metabolism of that nucleotide, either through inhibition of its synthesis (at the adenyl cyclase level), or through acceleration of its breakdown (at the phosphodiesterase level) (see Fig. 2).

Although pyrazole and MPC affect in the same way the hormone sensitive lipase activity, they influence in a different way the FFA re-esterification process. MPC decreases the esterification index both through enhancement of glycogen-synthetase activity and inhibition of phosphorylase activity in adipose cells.[21] Opposite to MPC, pyrazole enhances the re-esterification process both in basal and in stimulated lipolysis; this action is probably mediated through stimulation of the glycolytic pathway.

Finally, the time during which the action persists depends on the type of lipolysis considered. In basal conditions, the effects of both pyrazole and MPC are significantly reduced after 3 hr incubation. This time-depending activity may be attributed to the rebound-effect that follows the administration of most pyrazole-derivatives.[6]

When studying stimulated lipolysis, pyrazole shows no more antilipolytic effect after 3 hr, while MPC activity still persists; this discrepancy seems to be related to the higher antilipolytic activity of MPC.

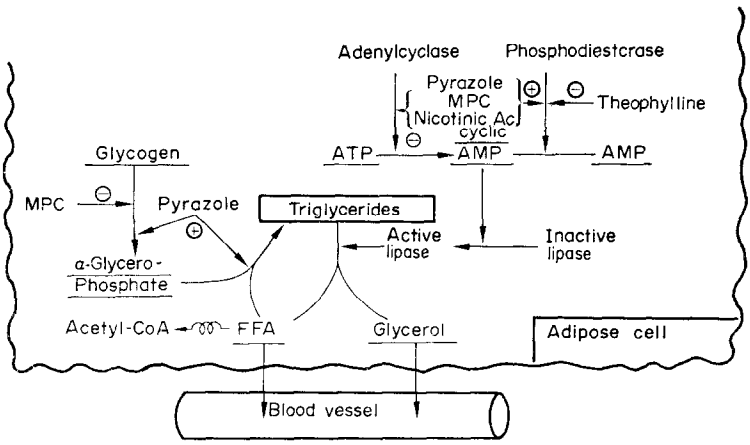

FIG. 2. Schematic representation of the regulatory mechanisms for the mobilization of FFA and glycerol from the stored triglycerides in adipose tissue, showing especially the different possible sites of action of pyazole and MPC. ⊖ and ⊕ indicate respectively inhibition and stimulation.

In conclusion, our results indicate that pyrazole does not reduce basal lipolysis, but significantly decreases glycerol and FFA release when lipolysis is stimulated. High concentrations of pyrazole are necessary however, to demonstrate this direct effect on stimulated lipolysis *in vitro*. Because of the toxicity of pyrazole,[22] only low doses of this compound can be administered *in vivo*. It seems therefore, that the effect of pyrazole on peripheral lipolysis does not play a significant part in the metabolic effects of pyrazole administration. This applies especially when studying the effect of pyrazole administration on the fatty liver induced by a large and single dose of ethanol, a condition which is known to stimulate peripheral lipolysis.[23] In this case, however, pyrazole could modify peripheral lipolysis by reducing the transformation of ethanol into acetaldehyde, which is known to enhance catecholamine secretion.[24]

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