control, received only normal saline i.p. The animals were weighed on the 16th day of administration and sacrificed by decapitation. The testes were excised, stripped of the tunica albuginea, weighed, chilled and processed according to Majumdar et al[12]. The supernatant of the testicular homogenate was then used for enzyme assay. The epididymis was also removed, freed from the adipose tissue, and weighed.

The 'marker' enzymes in cell differentiation during spermatogenesis, namely, acid phosphatase, hyaluronidase, 5'-nucleotidase, N-acetyl-B-glucosaminidase, B-galactosidase and UDPase were assayed according to the methods of Bergmeyer[13], Rhodes et al.[14], Huang and Keenan[15], Conchie[16], Lederberg[17], and Xuma and Turkington[10], respectively. Protein was estimated by the method of Lowry et al.[18].

Results and discussion. The rate of increase of body weight, the weight of the testes and epididymis were less in rats treated with $PGF_{2\alpha}$ than in the control. The differences were, however, insignificant. The table shows the specific activities of the 'marker' enzymes in testes in treated and control rats. The activity of acid phosphatase was higher ($p < 0.05$) in treated than in the control rats. Such increase in activity would mean an increase in the proacrosomal bodies on the administration of $PGF_{2\alpha}$[19]. The level of hyaluronidase in treated rats was significantly less ($p < 0.05$) than in the control. Hyaluronidase in testis is localized in acrosome of developing spermatids and mature spermatozoa[6]. A decrease in the level of hyaluronidase indicates suppression of the formation of acrosome in the newly formed spermatids. The activities of 5'-nucleotidase, N-acetyl-B-glucosaminidase, B-galactosidase and UDPase in the treated animals were not significantly different from that in the controls.

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Effects of several preoperative medications on fat cell lipolysis, and activity of adipose tissue cyclic AMP phosphodiesterase

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Summary. Effects were examined of atropine, diazepam, pethidene, promethazine, scopolamine, omnopon and papaverine on basal and noradrenaline-stimulated lipolysis in rat isolated fat cells and on rat adipose tissue cyclic AMP phosphodiesterase activity. Papaverine at high concentration (1 mM) inhibited both basal and hormone-stimulated lipolysis, whereas diazepam enhanced basal lipolysis. At a 'clinical dose', omnopon increased both basal and noradrenaline-stimulated lipolysis. Adipose tissue cAMP phosphodiesterase activity was strongly inhibited by 1 mM diazepam, papaverine, promethazine and omnopon (280 μg ml^{-1}). Lack of enhancement of lipolysis by the established cAMP phosphodiesterase antagonist papaverine, is compatible with simultaneous inhibition also of adipose adenyl cyclase. Diazepam-stimulated lipolysis is compatible with its phosphodiesterase inhibitory activity. It is proposed that papaverine-containing omnopon may offer some survival advantages during surgical stress by facilitating a caloric supply.

Human adipose tissue for study, in vitro, may be obtained by fat biopsy[3] or by harvesting available tissue from patients undergoing major surgery. This latter method provides large samples and is widely-used; yet little information is available regarding the effects of medications received by patients on the subsequent behaviour of their adipose tissue, when incubated in vitro.

The aim of this study was to investigate the effects of several commonly-used preoperative medications on adipose tissue lipolysis, using a rat isolated fat cell as a model; and to compare observed lipolytic activity with cyclic AMP (cAMP) phosphodiesterase activity, in view of the involvement of the cAMP-system in the lipolytic process.

Methods. Male Sprague-Dawley rats weighing 190–210 g were fasted overnight but allowed water ad libitum. They were sacrificed by a blow on the head and epididymal adipose tissue extirpated and used to prepare suspensions of isolated fat cells in Krebs-Ringer bicarbonate buffer solution (containing glucose 45 mg 100 ml^{-1} and bovine serum albumin 3.5 g 100 ml^{-1}) at pH 7.4 using a technique[4] modified from Rodbell[5].

The compounds examined were atropine (as the sulphate), diazepam, pethidine and promethazine, hydrochlorides, scopolamine hydrobromide and omnopon. Omnopon is a preparation of the hydrochlorides of the opium alkaloids, morphine representing about 50% of the total (and respon-

sible for the analgesic actions of opium) and papaverine is another component. The mixed nature of omnopon thus prevents its being assigned a precise molecular mass, and for this reason it was used on a weight/volume basis. Compounds were investigated in the absence and presence of noradrenaline (1×10^{-5} M) initially at 1×10^{-3} M, and subsequently at a concentration which would be achieved in the circulation in man following administration of a clinical dose. Since ethanol may inhibit adipose tissue lipolysis[6], stock solutions of all drugs were made in an equivolume mixture of methanol and water. Glycerol release during 90 min incubation at 37 °C was employed as the index of total lipolysis: it was estimated manually and related to the weight of intracellular lipid present[8].

The assay procedure for cyclic nucleotide phosphodiesterase activity[9], was modified from that of Brooker, Thomas and Appleman[10]. Fresh epididymal adipose tissue from fasted rats was homogenized in ice-cold tris-buffered sucrose (50 mM Tris, 250 mM sucrose) at pH 7.8 to yield an approximately 10% w/v homogenate. This was centrifuged at 10,000× g for 30 min at 4 °C and the crude supernatant (approximately 1.5 mg protein ml^{-1}) used as the enzyme source. Tris buffer, containing potential inhibitor compound (1×10^{-3} M), unlabelled cAMP, a tracer dose of tritiated compound (Radiochemical Centre, Amersham), 5′-AMP, and 5′-nucleotidase (10 U ml^{-1}) were pre-incubated with shaking for 2 min at 30 °C. The reaction was initiated by addition of 50 µl of enzyme preparation, and samples further incubated for 20 min. 1 ml of resin slurry (Bio-Rad AG1-x2-400 mesh) in water was added, and agitation continued for a further 10 min. Dioxan-based scintillation mixture (Koch-Light) was added, and the samples mixed and counted in a Beckman LS 100 spectrometer. Quenching was determined by a channel ratio method.

Table 1. Effects of pre-operative medications on basal and catecholamine-stimulated glycerol release from isolated fat cells

Compound	Concentration	Glycerol release NA present (1×10^{-5} M)	NA absent
Control	–	43.2±1.3	2.8±0.2
Solvent alone	–	46.4±1.9	4.1±0.4
Atropine	1×10^{-3} M	33.1±0.7	2.6±0.1
Diazepam		39.9±1.0	7.1±0.3***
Papaverine		4.5±0.2***	1.4±0.1**
Pethidine		31.2±3.7	2.1±0.1
Promethazine		33.1±0.8	3.1±0.1
Scopolamine		39.4±5.0	3.3±0.1
	(µM)		
Atropine	0.2	39.8±0.1	3.0±0.4
Diazepam	7.0	42.2±0.3	3.5±0.6
Papaverine	53.2	40.7±0.3	3.1±0.5
Pethidine	70.5	43.3±0.7	2.7±0.3
Promethazine	15.6	43.0±0.8	2.6±0.1
Scopolamine	0.2	44.7±0.3	3.0±0.4
Omnopon	4 µg ml^{-1}	56.7±3.1*	5.9±0.1*

Results are expressed as mean (of 3–5 experiments) ± SEM nmoles glycerol released mg^{-1} lipid per 90 min. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 2. Inhibitory effects of pre-operative medications (1×10^{-3} M) on adipose tissue cAMP phosphodiesterase activity

Compound	Inhibition (%)
Solvent mixture	26.6±4.0
Atropine	8.3±4.7
Diazepam	92.4±0.3*
Papaverine	98.7±0.8*
Pethidine	15.3±4.9
Promethazine	85.2±4.6*
Scopolamine	2.2±1.2
Omnopon**	70.5±1.5*

* $p<0.001$ (significance of elevation above solvent mixture value).
** Omnopon concentration 280 µg ml^{-1}.

Results. From table 1 it may be seen that the only significant effects on fat cell lipolysis were shown by diazepam and papaverine, but they were changes in different directions. Diazepam at 10^{-3} M caused a significant ($p<0.05$) increase of basal lipolysis, whereas papaverine reduced very significantly ($p<0.001$) both basal and catecholamine-stimulated glycerol release. No significant effects on lipolysis were observed when the compounds were examined at their clinical doses, except in the case of omnopon which increased both the basal and noradrenaline-stimulated lipolysis. In the cAMP phosphodiesterase assay, papaverine and diazepam were shown to be most potent inhibitors of the enzyme. Significant inhibition was also shown by promethazine and omnopon (at a concentration of 280 µg ml^{-1}, comparable to the diazepam dose used, on a weight/volume basis). The increase of adipose cell basal lipolysis by diazepam (table 1) is compatible with its action as an inhibitor of adipose cAMP phosphodiesterase (table 2). The lack of a further increment in the presence of noradrenaline indicates, probably, that intracellular cAMP concentration is already in excess of that required for maximal lipolysis. However, on this basis, cAMP-independent mechanisms of stimulation of lipolysis must be postulated in the case of omnopon[11].

Discussion. The observation of an inhibition of basal and hormonestimulated lipolysis by papaverine at 1×10^{-3} M is explained possibly by the recent observation that this drug, at high concentration, inhibits also adenyl cyclase[12]. Thus intracellular cAMP does not accumulate and phosphodiesterase inhibitory activity plays no role in enhancing lipolysis. Presumably the lower dose examined was beyond its range of activity for either adenyl cyclase or phosphodiesterase in this tissue. There exists also, however, the possibility of certain non-specific effects provoked by the drug concentrations used. Whether promethazine behaves in the same manner as papaverine, remains to be elucidated, although such explanation would be compatible with our observations.

With reservations relating to the interplay of α- and β-receptor agonists of human adipose tissue cells not prevailing in rat adipose cells, it seems possible that the choice of omnopon as a preoperative medication for patients undergoing major surgery, who may be fasted, fatigued and stressed, may offer certain survival advantages in facilitating a supply of free fatty acids from body calorie stores in adipose tissue. Similarly, this point should be considered when harvesting patients tissue for studies in vitro.

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