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The effect of α-adrenergic agonists on the membrane potential of fat-cell mitochondria *in situ*

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1. The accumulation of [^{3}H]methyltriphenylphosphonium by isolated fat-cells was used to estimate the membrane potential of mitochondria *in situ*. 2. An α-adrenergic receptor-mediated decrease in the apparent accumulation of [^{3}H]methyltriphenylphosphonium was observed. Methoxamine, clonidine and low concentrations of phenylephrine decreased the calculated mitochondrial membrane potential without significantly raising cyclic AMP levels, adenylate cyclase activity or stimulating lipolysis. The agonist potency order was phenylephrine > methoxamine > clonidine. 3. The decrease in the calculated mitochondrial membrane potential caused by phenylephrine, clonidine and methoxamine was blocked by the α-adrenergic antagonist prazosin but not by yohimbine nor by the β-antagonist propranolol. This suggests that the effect on the calculated mitochondrial membrane potential may be mediated by α_1-like receptors.

In the preceding paper (Davis & Martin, 1982) we demonstrated that β-adrenergic agonists cause a large decrease in the calculated membrane potential of fat-cell mitochondria *in situ* estimated from the accumulation of [^{3}H]TPMP$^+$. It was suggested that this was due to the uncoupling effect of non-esterified fatty acids released because of the stimulation of lipolysis. Phenylephrine has found wide use as a selective α-adrenergic agonist and was used in initial experiments to investigate whether α-adrenergic agonists affected the apparent accumulation of [^{3}H]TPMP$^+$ by fat-cells (Davis & Martin, 1982). Phenylephrine decreased the apparent accumulation of [^{3}H]TPMP$^+$ and this effect could be demonstrated with concentrations of phenylephrine as low as 0.1 μM. In order that this effect on the apparent accumulation of [^{3}H]TPMP$^+$ can be considered to be mediated by the α-adrenergic receptors, a large number of criteria must be satisfied. These criteria can be separated into two classes. The first consists of pharmacological evidence. If the effect of phenylephrine is an α-adrenergic response it should be mimicked by other α-adrenergic agonists, be blocked by α-adrenergic antagonists and be unaffected by β-adrenergic antagonists. The second consists of biochemical evidence that the response is not associated with the stimulation of adenylate cyclase.

The mechanism of action of β-adrenergic agonists causing the activation of adenylate cyclase is well understood (for review, see Fain, 1981). The mechanism of action of α-adrenergic agonists on the other hand is not so well characterized. In rat fat-cells reported α-adrenergic effects include the cyclic AMP-independent activation of glycogen phosphorylase and the inactivation of glycogen synthase (Lawrence & Larner, 1977, 1978), a transient activation of $^{42}K^+$ efflux (Perry & Hales, 1970), an increase in phospholipid labelling (Stein & Hales, 1972; Garcia-Sainz & Fain, 1980), an increase in the specific radioactivity of [^{32}P]ATP (Stein, 1975) and a possible increase in glucose transport (Luzio *et al.*, 1974).

In liver there is increasing evidence that α-adrenergic agonists increase the concentration of cytosolic free Ca^{2+} and that this mediates the effect of adrenaline on this tissue (Murphy *et al.*, 1980). The source of this Ca^{2+} is a matter of some controversy. In the early response the Ca^{2+} is thought to come from an internal source, which has been suggested to be mitochondrial by some groups (for review, see Exton, 1981). Recently two reports have indicated that there are stable changes in the Ca^{2+} metabolism by mitochondria isolated from the liver (Taylor *et al.*, 1980) and heart (Kessar &

Abbreviations used: $\Delta\psi_p$, plasma-membrane potential; $\Delta\psi_m$, mitochondrial membrane potential; $\Delta\bar{\mu}_{H^+}$, mitochondrial proton electrochemical gradient; ΔpH_m, mitochondrial pH gradient; TPMP$^+$, methyltriphenylphosphonium.

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Crompton, 1981) when these tissues are pre-treated with α-adrenergic agonists.

The purpose of these experiments was to determine whether α-adrenergic agonists affected the $\Delta\psi_m$ of mitochondria of isolated fat-cells. As α-adrenergic agonists appear to change the mitochondrial ion-balance it is possible that these changes will be reflected by the $\Delta\psi_m$.

Experimental

Materials

The sources of materials were the same as those described in the preceding paper (Davis & Martin, 1982). [2′,8′-^{3}H]Cyclic AMP (sp. radioactivity 52Ci/mmol), [2′-^{3}H]cyclic AMP (sp. radioactivity 26Ci/mmol) and [^{32}P]P_i were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. [α-^{32}P]ATP was prepared as described by Martin & Voorheis (1977). Yohimbine, phosphocreatine and creatine kinase were from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. Prazosin [1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-(2-furoyl)piperazine hydrochloride] was a gift from Pfizer, Sandwich, Kent, U.K. Methoxamine [2-amino-1-(2,5-dimethoxyphenyl)-propan-1-ol hydrochloride] was from Burroughs Wellcome, London, U.K. Clonidine [2-(2,6-dichloroanilino)-2-imidazoline hydrochloride] was a gift from Boehringer, Ingelheim, Germany. ATP (grade 1), dithiothreitol, the enzymes and coenzymes for glycerol assays and all other biochemicals were from Boehringer, Mannheim, Germany. Percoll was bought from Pharmacia Fine Chemicals, Uppsala, Sweden. All other reagents were of analytical grade.

Preparation of fat cells and the determination of $^{86}Rb^+$ and [^{3}H]TPMP$^+$ accumulation by isolated fat-cells

Fat-cells were isolated from rat epididymal fat-pads and the accumulation of $^{86}Rb^+$ and [^{3}H]-TPMP$^+$ by the isolated fat-cells was estimated as described in the preceding paper (Davis & Martin, 1982).

Preparation of fat-cell plasma membranes

Plasma membranes were prepared by a minor modification of the Percoll method described by Belsham *et al.* (1980) from isolated fat-cells. EGTA was omitted from the buffers used during the cell fractionation and the isolated membranes were washed three times with 1mM-$NaHCO_3$. The membranes were stored in liquid N_2 before use.

Glycerol assays

Fat-cell suspensions were deproteinized by the addition of an equal volume of 10% (w/v) trichloroacetic acid. The samples were then centrifuged and the supernatants removed to be extracted three times with 5vol. of diethyl ether. Glycerol was assayed by the method of Garland & Randle (1962).

Cyclic AMP assays

A portion (1ml) of a fat cell suspension was deproteinized by the addition of 30μl of 3M-$HClO_4$. All subsequent steps were performed at 0°C. The infranatant was neutralized with 1M-triethanolamine/KOH buffer, pH 14, and then centrifuged to give a supernatant that was used for the assay of cyclic AMP by the binding assay of Brown *et al.* (1972). Bovine adrenal binding protein was a gift from J. P. Moore of this department and was prepared as described by Brown *et al.* (1972). [2′,8′-^{3}H]Cyclic AMP (1pmol; sp. radioactivity 52Ci/mmol) was used in each incubation with the binding protein and the sample. A standard curve was prepared during each experiment with known amounts of unlabelled cyclic AMP from 0 to 40pmol.

Adenylate cyclase assays

The adenylate cyclase activity of isolated fat-cell membranes was assayed by the method of Salomon *et al.* (1974). Incubations were carried out for 20min at 30°C in a total volume of 0.1ml containing 25mM-Tris/HCl (pH 7.4), 1mM-dithiothreitol, 10mM-$MgCl_2$, 0.1mM-cyclic AMP, 0.5mM-[α-^{32}P]-ATP (10Ci/mol), 0.1mM-GTP, 5mM-phosphocreatine and 5 units of creatine kinase with other additions as stated. The results are expressed as nmol of cyclic AMP formed/mg of protein per min. Protein was measured in the isolated membranes by the method of Lowry *et al.* (1951).

Results

Pharmacological evidence for an α-receptor-mediated effect on the apparent [^{3}H]TPMP$^+$ accumulation by isolated fat-cells

Three α-selective agonists were investigated: methoxamine, clonidine and phenylephrine. It was found that low concentrations of all three of these agonists resulted in a lower apparent accumulation of [^{3}H]TPMP$^+$ than by control cells (Fig. 1*a*), but had no effect on the accumulation of $^{86}Rb^+$ (results not shown). The order of potency of these agonists was phenylephrine > methoxamine > clonidine. Recently α-receptors have been classified as α_1 or α_2 according to their pharmacological properties (Langer, 1974; Berthelson & Pettinger, 1977; U'Pritchard *et al.*, 1978; Hoffman *et al.*, 1979, 1980; Wood *et al.*, 1979). The order of potency of the agonists to give the effect reported here is similar to that observed on postsynaptic (α_1) receptors (Starke *et al.*, 1975).

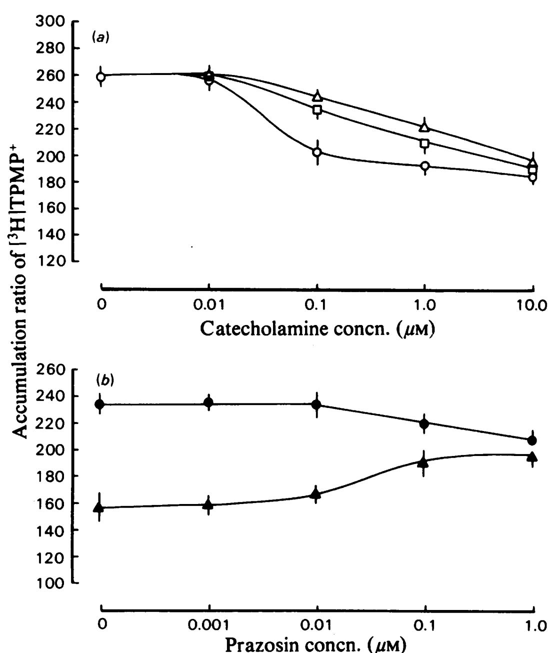

Fig. 1. *Effect of α-adrenergic agonist and antagonist concentration on the accumulation of* [^{3}*H*]*TPMP*$^+$

The accumulation ratio of [^{3}H]TPMP$^+$ after 40min of incubation was measured as described in the Experimental section. The results are those of one representative experiment and are expressed as means of three separate incubations. The bars indicate the standard deviation. (*a*) shows the effect of agonist concentration. Similar results were obtained with four other preparations of fat-cells. Symbols: ○, phenylephrine; △, clonidine; □, methoxamine. (*b*) shows the effect of prazosin concentration in the presence and absence of 10μM-methoxamine. Similar results were obtained with three other preparations of fat-cells. Symbols: ●, control; ▲, 10μM-methoxamine.

Two α-adrenergic antagonists were used to try to block the effect of the three agonists. Prazosin was found to be a very potent antagonist of all three agonists (Fig. 1*b*, Table 1). However, yohimbine was not found to be effective (Table 2). As prazosin is 10000-fold more potent on α_1- than on α_2-receptors (U'Pritchard *et al.*, 1978; Hoffman *et al.*, 1979) and yohimbine is 500-fold more potent on α_2- than on α_1-receptors (Hoffman *et al.*, 1979), this provides further evidence that the adrenergic effect on the accumulation of [^{3}H]TPMP$^+$ may be related to α_1-like receptors. In particular it demonstrates that the α_2-selective agonist clonidine (Starke *et al.*, 1975; Drew, 1977, 1978) may be acting as an α_1-agonist to give the effect on the apparent [^{3}H]TPMP$^+$ accumulation, as it is blocked by prazosin at low concentrations and not by yohimbine.

Propranolol is a *β*-adrenergic antagonist and would be expected to block the effect of methoxamine, clonidine and phenylephrine if they were acting as weak β-agonists to give their effect. It was found that propranolol had no effect on the action of the three agonists except phenylephrine at high concentrations (Table 3).

Biochemical evidence for an α-receptor-mediated effect on the apparent accumulation of [^{3}*H*]*TPMP*$^+$ *by fat-cells*

If the α-agonists were acting as weak *β*-agonists to give the effect on the apparent [^{3}H]TPMP$^+$ accumulation they should do so by raising the level of cyclic AMP in the cells. Cyclic AMP levels in the cells and medium were measured in cells treated with 5μM-isoprenaline or 10μM-phenylephrine (Fig. 2). Isoprenaline substantially increased the amount of cyclic AMP, but phenylephrine was found to increase the levels only slightly above the control and this increase was not significant. The small

Table 1. *Effect of prazosin on the accumulation of* [^{3}*H*]*TPMP*$^+$ *by fat-cells*

Fat-cells were pre-incubated for 30min at 37°C in a shaking water bath before 50μM-^{86}RbCl (0.2μCi/ml) and 0.3μM-[^{3}H]TPMP$^+$ (0.5μCi/ml) were added. ^{86}Rb$^+$ and [^{3}H]TPMP$^+$ accumulation ratios were measured after 40min of incubation as described in the Experimental section. The results are those from one representative experiment and are expressed as means of three separate incubations ± S.D. Similar results were obtained with three other preparations of fat-cells. The mitochondrial membrane potential was calculated as described by Davis *et al.* (1981) with the assumption that the rubidium diffusion potential is in equilibrium with the plasma-membrane potential.

	^{86}Rb$^+$ accumulation ratio	[^{3}H]TPMP$^+$ accumulation ratio	Calculated mitochondrial potential
Control	18.4 ± 0.4	237.2 ± 9.9	163.1
Prazosin (1μM)	18.2 ± 0.3	215.7 ± 7.0	160.5
Phenylephrine (1μM)	18.5 ± 0.4	167.3 ± 7.3	151.9
Phenylephrine (1μM) + 1μM-prazosin	18.3 ± 0.2	203.3 ± 10.9	158.5
Clonidine (10μM)	17.8 ± 0.3	177.2 ± 13.1	155.0
Clonidine (10μM) + 1μM-prazosin	18.1 ± 0.2	201.4 ± 7.2	158.5
Methoxamine (10μM)	17.9 ± 0.5	151.6 ± 5.5	149.7
Methoxamine (10μM) + 1μM-prazosin	18.2 ± 0.2	196.1 ± 8.0	157.5

Table 2. *Effect of yohimbine on the accumulation of [^3H]TPMP$^+$ by fat-cells*
The details of the experimental procedures were the same as those described in the legend to Table 1. The results are those from one representative experiment and are expressed as means of three separate incubations ± S.D. Similar results were obtained with three other preparations of fat-cells.

	$^{86}Rb^+$ accumulation ratio	[^3H]TPMP$^+$ accumulation ratio	Calculated mitochondrial potential
Control	17.4 ± 0.3	251.2 ± 4.3	166.6
Yohimbine (1 μM)	17.1 ± 0.4	232.8 ± 8.6	164.8
Phenylephrine (1 μM)	17.6 ± 0.2	191.7 ± 1.6	157.9
Phenylephrine (1 μM) + 1 μM-yohimbine	17.3 ± 0.5	196.7 ± 10.2	159.2
Clonidine (10 μM)	17.7 ± 0.5	204.7 ± 5.7	159.8
Clonidine (10 μM) + 1 μM-yohimbine	17.1 ± 0.4	211.6 ± 5.6	161.9
Methoxamine (10 μM)	17.9 ± 0.6	189.4 ± 10.1	157.0
Methoxamine (10 μM) + 1 μM-yohimbine	17.6 ± 0.4	185.4 ± 7.4	156.8

Table 3. *Effect of propranolol on the accumulation of [^3H]TPMP$^+$ by fat-cells*
The details of the experimental procedures were the same as those described in the legend to Table 1. The results are those from one representative experiment and are expressed as means of three separate incubations ± S.D. Similar results were obtained with three other preparations of fat-cells.

	$^{86}Rb^+$ accumulation ratio	[^3H]TPMP$^+$ accumulation ratio	Calculated mitochondrial potential
Experiment 1			
Control	15.4 ± 0.3	243.8 ± 5.5	169.3
Propranolol (1 μM)	14.9 ± 0.4	226.7 ± 8.5	168.1
Isoprenaline (0.1 μM)	14.0 ± 0.4	88.3 ± 8.5	139.4
Isoprenaline (0.1 μM) + 1 μM-propranolol	14.3 ± 0.4	179.6 ± 10.0	162.3
Phenylephrine (0.1 μM)	15.0 ± 0.3	191.7 ± 9.6	162.8
Phenylephrine (0.1 μM) + 1 μM-propranolol	14.8 ± 0.4	190.0 ± 12.2	163.0
Phenylephrine (1 μM)	15.6 ± 0.5	180.1 ± 4.2	159.7
Phenylephrine (1 μM) + 1 μM-propranolol	15.3 ± 0.2	174.3 ± 6.8	159.2
Phenylephrine (100 μM)	15.4 ± 0.2	152.1 ± 7.7	154.8
Phenylephrine (100 μM) + 1 μM-propranolol	15.0 ± 0.4	174.8 ± 7.9	160.0
Experiment 2			
Control	17.2 ± 0.4	249.4 ± 7.2	166.7
Propranolol (1 μM)	17.6 ± 0.4	235.8 ± 6.4	164.3
Clonidine (10 μM)	17.1 ± 0.3	192.6 ± 8.4	158.9
Clonidine (10 μM) + 1 μM-propranolol	17.4 ± 0.5	190.2 ± 9.2	158.0
Methoxamine (10 μM)	17.3 ± 0.2	187.4 ± 7.9	157.7
Methoxamine (10 μM) + 1 μM-propranolol	17.4 ± 0.3	183.6 ± 10.0	156.9

increase observed with phenylephrine may be sufficient to activate lipolysis because of the possible compartmentalization of the cyclic AMP. To further investigate the β-potency of the α-agonists used in these experiments two types of experiments were done. First, lipolysis by the fat-cells was measured as glycerol release and, secondly, the effect of the agonists on the activity of adenylate cyclase in isolated plasma membranes was investigated.

The release of glycerol by fat-cells treated with the α-selective agonists is presented in Fig. 3(*a*). Methoxamine and clonidine did not stimulate glycerol release but phenylephrine did stimulate glycerol release at high concentrations. Phenylephrine stimulated glycerol release at 10 μM- and 100 μM-phenylephrine was as potent as 0.1 μM-isoprenaline. Therefore the effect of phenylephrine on the accumulation of [^3H]TPMP$^+$ at these high concentrations is probably due to the stimulation of lipolysis. However, an effect of phenylephrine on the accumulation of [^3H]TPMP$^+$ was observed at 0.1 μM (Fig. 1*a*), which was inhibitable with prazosin, an α-antagonist (Table 1), but not with propranolol, a β-antagonist (Table 3). It is likely that the effects of low concentrations of phenylephrine are an α-response, but that at concentrations of 10 μM and above the β-response becomes important. The effects of these high concentrations of phenylephrine on the

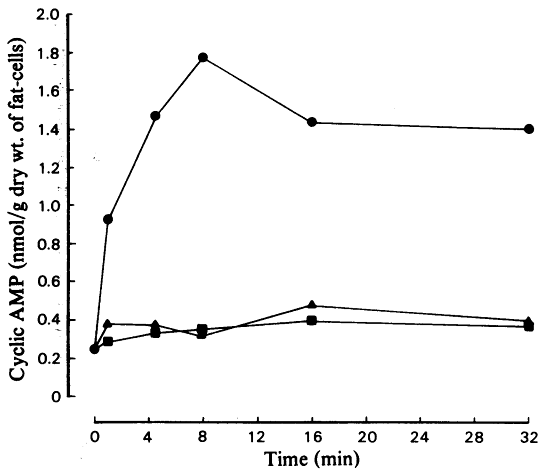

Fig. 2. *Cyclic AMP levels in fat-cells treated with isoprenaline or phenylephrine*

Cyclic AMP levels after different times of incubation were measured as described in the Experimental section. The results are expressed as means of three separate preparations of fat-cells. Symbols: ■, control; ▲, 10μM-phenylephrine; ●, 5μM-isoprenaline.

accumulation of [^{3}H]TPMP$^+$ could be partially blocked with propranolol (Table 3). A weak β-effect of phenylephrine at high concentrations has been suggested by Lawrence & Larner (1977) and the data here confirm this. The order of agonist potency for the stimulation of glycerol release was isoprenaline ⋙ phenylephrine ≫ methoxamine > clonidine.

The stimulation of adenylate cyclase in isolated plasma membranes by α-selective agonists is presented in Fig. 3(*b*). GTP (0.1 mM) was included in the incubations to ensure the maximal effects of the agonists (Rodbell *et al.*, 1975). The results show a marked resemblance to the dose–response curve for the glycerol release (Fig. 3*a*). Clonidine and methoxamine had little effect on the adenylate cyclase activity in the presence of 0.1 mM-GTP. The α_2-inhibition of adenylate cyclase by clonidine, which might be present in rat fat-cell plasma membranes (by analogy with hamster fat-cells), would not be expected to be observed under the conditions used in these experiments. To demonstrate the α_2-inhibition in other tissues, such as hamster fat-cells or platelets, high concentrations of sodium are required (for review, see Jakobs *et al.*, 1980). The order of potency of the agonists to stimulate the adenylate cyclase activity was isoprenaline > phenylephrine ≫ methoxamine = clonidine.

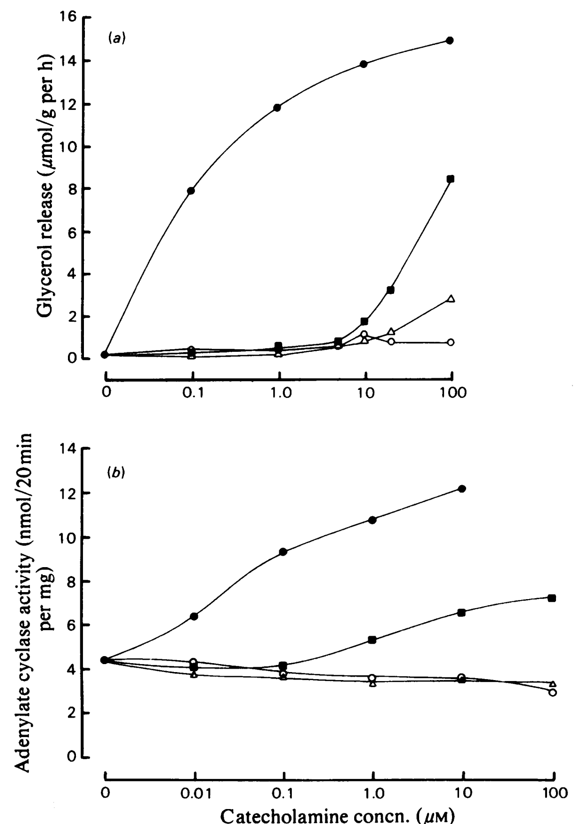

Fig. 3. *Effect of adrenergic agonists on glycerol release by fat-cells and the activity of adenylate cyclase in isolated plasma membranes*

(*a*) shows the effect of agonists on glycerol release by isolated fat-cells. Glycerol release was measured as described in the Experimental section. Results are expressed as means of four experiments on separate preparations of fat-cells. Symbols: ●, isoprenaline; ■, phenylephrine; ○, clonidine; △, methoxamine. (*b*) shows the effect of agonist concentration on the activity of adenylate cyclase in isolated fat-cell plasma membranes. Adenylate cyclase activity was measured as described in the Experimental section. Results are expressed as means of four experiments. Symbols: ●, isoprenaline; ■, phenylephrine; ○, clonidine; △, methoxamine.

Time course of the effect of methoxamine on the apparent [^{3}H]TPMP$^+$ accumulation ratio

The time course of the effect of methoxamine on glycogen metabolism was investigated by Lawrence & Larner (1978). Phosphorylase was maximally activated after 1 min of incubation with methoxamine and then gradually declined with further incubation. The effect of methoxamine on glycogen synthase was maximal after 4 min and was maintained with more prolonged incubation. Routinely the effect of methoxamine on the apparent [^{3}H]-TPMP$^+$ accumulation ratio was investigated 40 min after the addition of the agonist to ensure complete equilibration of the [^{3}H]TPMP$^+$. Therefore the effect

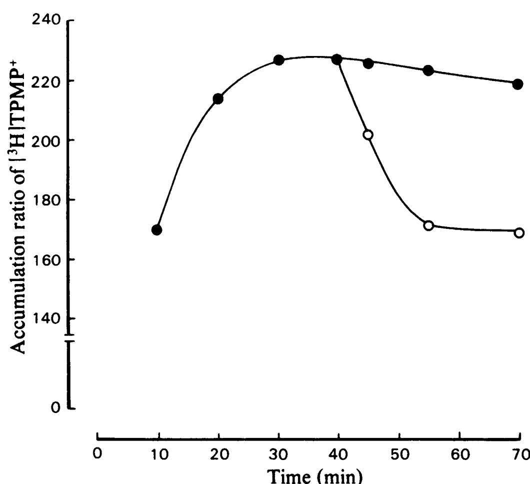

Fig. 4. *Time course of the effect of methoxamine on the apparent [^{3}H]TPMP$^+$ accumulation by fat-cells*
The accumulation ratio of [^{3}H]TPMP$^+$ was measured as described in the Experimental section. Methoxamine (10μM) was added at 40min after the start of the incubation with [^{3}H]TPMP$^+$. The results are those of one representative experiment and are expressed as means of three separate incubations. Similar results were obtained with one other preparation of fat-cells. Symbols: ●, control; ○, 10μM-methoxamine.

of methoxamine on the apparent [^{3}H]TPMP$^+$ accumulation ratio could be either a direct effect of the methoxamine or the response of the fat-cells to recover after methoxamine stimulation. To resolve these two possibilities the effect of 10μM-methoxamine was investigated on the [^{3}H]TPMP$^+$ accumulation of fat-cells pre-equilibrated with [^{3}H]-TPMP$^+$ (Fig. 4). An effect of methoxamine was observed 5min after the addition of the agonist. However, because of the slow equilibration time of TPMP$^+$ the maximum response was not observed until 15min after the addition of methoxamine. The slow response of TPMP$^+$ to changes in cellular membrane potentials makes the interpretation of this experiment difficult, but it does appear that the change in the apparent accumulation ratio of [^{3}H]TPMP$^+$ caused by methoxamine is an early event in the response of the fat-cells.

Discussion

α-Adrenergic effects on the apparent [^{3}H]TPMP$^+$ accumulation

The possibility that there might be an α-adrenergic effect on the accumulation of [^{3}H]TPMP$^+$ is complicated by the presence of a high number of β-receptors on rat fat-cell plasma membranes. Phenylephrine, an α-agonist that is commonly used, has been shown to markedly stimulate lipolysis and adenylate cyclase activity at high concentrations (Fig. 3). Therefore it cannot be concluded that the phenylephrine at these concentrations is acting only as an α-agonist as it appears to have some β-activity as well. Methoxamine is a better α-selective agonist, as it stimulated lipolysis very weakly at concentrations of 100μM. However, clonidine and low concentrations of methoxamine and phenylephrine did not stimulate glycerol release or adenylate cyclase activity, but still decreased the apparent accumulation of [^{3}H]TPMP$^+$ by fat-cells. Furthermore the effects of clonidine and low concentrations of methoxamine and phenylephrine were prevented by prazosin (a potent α_1-selective antagonist) and were not blocked by propranolol (a β-antagonist). These results indicate that there may be an α-effect on the apparent accumulation of [^{3}H]TPMP$^+$ that is independent of the β-receptors, the stimulation of adenylate cyclase and the stimulation of lipolysis. The response is probably receptor-mediated rather than a non-specific action of the agonist, because it was given by three different agonists and was inhibited by a specific antagonist. The potency order of the agonists and the finding that prazosin is a very effective antagonist, whereas yohimbine is a poor antagonist, suggests that the receptors mediating the response are similar to α_1-receptors.

The nature of the α-adrenergic receptors of rat fat-cells has been investigated by Giudicelli *et al.* (1981) by a direct binding technique. It was found that the α-receptors of rat fat-cells are unusual and do not correspond to the α_1- and α_2-receptor sub-types that are found in hamster fat-cells (Pecquery & Giudicelli, 1980). Further work is therefore necessary to characterize the α-adrenergic receptors of rat fat-cells in order to determine what receptors are responsible for the effect reported here.

α-Adrenergic effects on the calculated cell membrane potentials

The decrease in the [^{3}H]TPMP$^+$ accumulation when fat-cells were incubated with α-adrenergic agonists can be interpreted in many ways. The cause could be a decrease in the mitochondrial volume, an increase in the cellular relative activity coefficient of [^{3}H]TPMP$^+$, a decrease in the plasma membrane potential or a change in the mitochondrial membrane potential. A decrease in the mitochondrial volume is possible, but a mechanism for this does not seem to be readily apparent. To account for the effect at 10μM-methoxamine the mitochondria would have to shrink to about 70% of their volume in control cells. A change in the relative activity coefficient of the [^{3}H]TPMP$^+$ could occur if the

[^{3}H]TPMP^{+} bound to the agonist or if the metabolic products of α-adrenergic stimulation either bound to the [^{3}H]TPMP^{+} or affected the way in which the [^{3}H]TPMP^{+} bound to some cellular or medium constituent. The agonist itself does not change the relative activity coefficient of the [^{3}H]-TPMP^{+} as the effect is given by more than one agonist and can be blocked by a specific α-adrenergic antagonist. However, it is possible that some metabolic product of α-adrenergic stimulation does change the activity coefficient and this must be borne in mind when considering other explanations of the α-adrenergic effect on the [^{3}H]TPMP^{+} accumulation, such as a change in the mitochondrial membrane potential.

A cause of the α-adrenergic effect on the [^{3}H]TPMP^{+} accumulation could be that the agonists depolarized the plasma membranes. To account for the effect of 10 μM-methoxamine the plasma membrane would have to be depolarized by about 12 mV. We are not aware of any measurements of α-adrenergic effects on the plasma-membrane potential of isolated white fat-cells. Petrozzo & Zierler (1976) have demonstrated that adrenaline depolarizes the plasma membrane of fat-cells by using a fluorescent probe. However, this effect appears to be mediated by β-adrenergic receptors as it was blocked by β-antagonists but not by α-antagonists. Perry & Hales (1970) have reported that the efflux of K^{+} is stimulated in fat-cells during α-adrenergic stimulation. In liver it has been reported that the stimulation of K^{+} efflux can be blocked by quinine. This observation was interpreted by Burgess *et al.* (1979) as indicating that there is a Ca^{2+}-activated K^{+} channel in liver membranes. It might therefore be expected that the increase in K^{+} conductance of the plasma membrane may lead to a hyperpolarization (Haylett & Jenkinson, 1972). In brown fat-cells there is evidence for an α- as well as a β-adrenergic-dependent depolarization of the plasma membrane, but this is a very specialized tissue that is involved in thermogenesis and may have different responses to white fat-cells (Fink & Williams, 1976).

Finally the effect on the [^{3}H]TPMP^{+} accumulation may be caused by a change in the membrane potential of one of the internal organelles, such as the mitochondria. The calculation of the mitochondrial membrane potential from the accumulation of [^{3}H]TPMP^{+} assumes that the mitochondria are responsible for all the potential dependent accumulation of [^{3}H]TPMP^{+} by cellular organelles. The justification for this assumption is discussed by Deutsch *et al.* (1979), Hoek *et al.* (1980), Scott & Nicholls (1980) and Davis *et al.* (1981). If the nucleus or endoplasmic reticulum contributed significantly to the accumulation of the [^{3}H]TPMP^{+} then a depolarization of one of these organelles might account for the α-adrenergic effect.

α-Adrenergic changes in the mitochondrial membrane potential

If the α-adrenergic effect on the accumulation of [^{3}H]TPMP^{+} by isolated fat-cells is caused by a change in the mitochondrial membrane potential this would be a novel effect of these agonists on the mitochondria of fat-cells. A mechanism for reducing the mitochondrial membrane potential is an increase in the rate of ion movements across the mitochondrial inner membrane (for example, an increase in the proton translocation by the ATP synthase). We have no evidence for which ions might be involved, except that it is not likely to be the uncoupling effect of non-esterified fatty acids, which are probably the cause of the depolarization observed with β-adrenergic agonists.

In liver it has been demonstrated that α-adrenergic stimulation leads to an increase in the concentration of free Ca^{2+} in the cytoplasm (Murphy *et al.*, 1980) and it has been suggested that this mediates the effect of the hormone on glycogenolysis. Exton (1981) has proposed that the mitochondria are the source of this Ca^{2+} for periods up to 5 min after hormone addition when the stimulation of glycogen phosphorylase is maximal [but see Althaus-Salzmann *et al.* (1980) and Poggioli *et al.* (1980)]. After this time there is an increase in the Ca^{2+} uptake across the plasma membrane and into the mitochondria, which is associated with the termination of the hormonal signal. If Ca^{2+} were to be released from the mitochondria it would be against the electrochemical gradient across the mitochondrial inner membrane because of the high mitochondrial membrane potential (Mitchell & Moyle, 1969). Therefore Ca^{2+} release has to be an active transport process and in liver the efflux pathway is linked to an electroneutral proton antiport carrier (for review, see Nicholls & Crompton, 1980). As the uptake and efflux pathways work simultaneously in isolated mitochondria, net release of Ca^{2+} from the mitochondria could be achieved by either inhibition of the uptake pathway or activation of the efflux pathway.

Stable changes in the mitochondrial Ca^{2+} metabolism caused by α-adrenergic agonists that persist during the isolation of mitochondria have been reported. Taylor *et al.* (1980) have demonstrated that mitochondria isolated from phenylephrine-treated livers have higher rates of Ca^{2+} uptake than mitochondria isolated from control livers. This effect may be the result of the higher respiration rates observed in mitochondria from phenylephrine-treated livers (Taylor *et al.*, 1980) rather than an increase in the activity of the Ca^{2+} uptake pathway because the Ca^{2+} was measured at saturating concentrations of Ca^{2+} (100 μM). Under these

conditions the rate of Ca^{2+} uptake is limited by the rate of respiration (for review, see Saris & Akerman, 1979). However, Taylor *et al.* (1980) have suggested that this increased uptake of Ca^{2+} may be responsible for the restoration of normal mitochondrial Ca^{2+} content after α-adrenergic stimulation. Recently, Kessar & Crompton (1981) reported that there was an α-adrenergic stimulation of Ca^{2+} uptake by isolated heart mitochondria. This stimulation was shown to be primarily due to a decrease in the apparent K_m of the Ca^{2+} uptake pathway for extramitochondrial Ca^{2+} and to be an early event of α-adrenergic stimulation. It is therefore possible that the α-adrenergic response of the heart may be different to that of liver. If the stimulation of Ca^{2+} cycling across the mitochondrial inner membrane as suggested by Kessar & Crompton (1981) for heart muscle also occurred in fat-cells it would account for the decrease in the calculated mitochondrial membrane potential reported in the present paper.

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