Effects of verapamil on lipolysis due to dibutyryl cyclic AMP

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The effects of verapamil, a calcium antagonist, on lipolysis in isolated rat adipocytes were studied. Verapamil (100 μM) potentiated lipolysis due to dibutyryl cyclic AMP (Bt_2cAMP) at submaximal concentrations, with or without extracellular Ca^{2+} . Lipolysis due to 0.5 mM- Bt_2cAMP was potentiated by verapamil in a dose-dependent manner up to 200 μM , whereas at concentrations higher than 100 μM the stimulatory effect of verapamil was progressively diminished with or without extracellular Ca^{2+} . Verapamil showed only an inhibitory effect on lipolysis due to adrenaline (0.1–10 μM) or 3-isobutyl-1-methylxanthine (IBMX; 25–200 μM). The stimulatory effect of verapamil on lipolysis due to Bt_2cAMP was not blocked by α -adrenergic antagonists. These results suggest (i) that verapamil has a biphasic effect on lipolysis due to Bt_2cAMP and only an inhibitory effect on that due to adrenaline or IBMX, and (ii) that extracellular Ca^{2+} or α -adrenergic receptors are not involved in the action of verapamil.

Although it is generally accepted that lipolysis is stimulated by a rise in intracellular cyclic AMP concentration (Butcher *et al.*, 1965; Weiss *et al.*, 1966), it has been pointed out that there is discrepancy between intracellular cyclic AMP concentrations and the extent of hormone-induced lipolysis (Lang & Schwyzer, 1972; Wise & Jungas, 1977; Fain *et al.*, 1979; Gaion & Krishna, 1982). Several lines of evidence indicate the involvement of Ca^{2+} in lipolysis (Mosinger & Vaughan, 1967; Schimmel, 1973; Siddle & Hales, 1974). Rasmussen (1980) has suggested that Ca^{2+} and cyclic AMP function as interrelated messengers in determining cellular response and that these two messengers almost always act together.

Verapamil is commonly used as a calcium antagonist (Fleckenstein, 1977). However, verapamil has been found to interact with α -adrenergic receptors in rat heart and brain (Glossman & Hornung, 1980; Frelin *et al.*, 1982), rat liver (Blackmore *et al.*, 1979), and human platelets (Barnathan *et al.*, 1982).

In the present study we investigated the effects of verapamil on lipolysis in isolated rat adipocytes. Verapamil showed a biphasic effect on lipolysis

due to Bt_2cAMP , with or without extracellular Ca^{2+} , and this effect was not blocked by α -adrenergic antagonists.

Experimental

Materials

Bovine serum albumin (non-esterified-fatty-acid-free, fraction V), crude collagenase (type I), adrenaline (epinephrine), cyclic AMP and Bt_2cAMP were purchased from Sigma. IBMX and dibenamine were from Nakarai Chemicals, Kyoto, Japan. Phenoxybenzamine was from Tokyo Kasei, Tokyo, Japan. Yohimbine was from Wako Pure Chemicals, Osaka, Japan. Verapamil was a gift from Eisai, Tokyo, Japan.

Methods

Isolation of adipocytes. Adipocytes were isolated from the epididymal fat-pads of male Sprague-Dawley rats (180–230 g) by the method of Rodbell (1964) and as previously described (Goko *et al.*, 1981), except that Ca^{2+} was omitted from the medium.

Lipolysis. Isolated adipocytes were incubated for 60 min at 37°C under O_2/CO_2 (19:1) with various concentrations of Bt_2cAMP , IBMX, adrenaline or α -adrenergic antagonists in the presence or ab-

Abbreviations used: Bt_2cAMP , dibutyryl cyclic AMP; IBMX, 3-isobutyl-1-methylxanthine.

sence of verapamil in Ca^{2+} -free Krebs-Ringer bicarbonate buffer [119 mM-NaCl/4.8 mM-KCl/1.2 mM/ KH_2PO_4 /1.2 mM- MgSO_4 /25 mM- NaHCO_3 /bovine serum albumin (30 mg/ml)] supplemented with 10 μM -EGTA for Ca^{2+} -minus medium and with 1.3 mM- CaCl_2 for Ca^{2+} -plus medium. Lipolysis was determined by measuring glycerol release into the medium.

Results

As shown in Fig. 1, lipolysis was enhanced by Bt_2cAMP in a dose-dependent manner in the absence or presence of 1.3 mM- Ca^{2+} in the medium. The addition of 100 μM -verapamil led to leftward shifts of dose-response curves; i.e., concentrations of Bt_2cAMP for half-maximal activation

Fig. 1. Effect of verapamil on lipolysis due to Bt_2cAMP or cyclic AMP

Isolated adipocytes were incubated for 60 min at 37°C with the indicated concentrations of Bt_2cAMP or cyclic AMP in the presence or absence of 100 μM -verapamil in Ca^{2+} -minus (10 μM -EGTA) medium (a) or 1.3 mM- Ca^{2+} -plus medium (b). Results are means $\pm$ S.E.M. for triplicate determinations from three (Bt_2cAMP) or two (cyclic AMP) separate experiments. ●, Bt_2cAMP ; ○, Bt_2cAMP + 100 μM -verapamil; ▲, cyclic AMP; △, cyclic AMP + 100 μM -verapamil.

were shifted from 1.0 mM to 0.3 mM in the Ca^{2+} -plus (1.3 mM) medium and from 1.2 mM to 0.4 mM in the Ca^{2+} -minus (EGTA 10 μM) medium. Verapamil did not potentiate the basal lipolysis. The maximal lipolysis due to 5 mM- Bt_2cAMP was slightly inhibited by 100 μM -verapamil. Cyclic AMP (0.25–5 mM) had little effect on lipolysis, and the addition of 100 μM -verapamil did not potentiate lipolysis.

Fig. 2 shows that lipolysis due to 0.5 mM- Bt_2cAMP was potentiated by the addition of verapamil in a dose-dependent manner up to 200 μM in the presence or absence of 1.3 mM- Ca^{2+} . However, the stimulatory effect of verapamil progressively diminished at concentrations more than 200 μM . Thus verapamil affects lipolysis due to Bt_2cAMP in a biphasic manner.

Next, we investigated the effects of verapamil on lipolysis due to adrenaline and IBMX in the Ca^{2+} -minus (EGTA 10 μM) medium. In contrast with that due to Bt_2cAMP , lipolysis due to adrenaline or IBMX at all concentrations tested was inhibited by the addition of 100 μM -verapamil (Figs. 3 and 4). High concentrations of adrenaline or IBMX did not overcome the inhibitory effect of verapamil.

Adipocyte cells have α -adrenergic receptors (Meisner & Carter, 1977). In order to see whether verapamil exerts these effects by the interaction with α -adrenergic receptors, we examined the effects of α -adrenergic antagonists on lipolysis. As

Fig. 2. Dose-response curves for the stimulation of lipolysis due to 0.5 mM- Bt_2cAMP by verapamil

Isolated adipocytes were incubated for 60 min at 37°C with 0.5 mM- Bt_2cAMP and the indicated concentrations of verapamil in the absence (□) (10 μM -EGTA) or presence (■) of 1.3 mM- Ca^{2+} . Results are means for triplicate determinations from two separate experiments.

Fig. 3. *Effect of verapamil on lipolysis due to adrenaline* Isolated adipocytes were incubated for 60 min at 37°C with the indicated concentrations of adrenaline without (●) or with (○) 100 μM-verapamil in Ca²⁺-minus (10 μM-EGTA) medium. Results are means ± S.E.M. for triplicate determinations from three separate experiments.

Fig. 4. *Effect of verapamil on lipolysis due to IBMX* Isolated adipocytes were incubated for 60 min at 37°C with the indicated concentrations of IBMX without (●) or with (○) 100 μM-verapamil in Ca²⁺-minus (10 μM-EGTA) medium. Results are means ± S.E.M. for triplicate determinations from three separate experiments.

shown in Fig. 5, none of these agents affected lipolysis due to 0.5 mM-Bt₂cAMP or potentiation of lipolysis by 25 μM-verapamil.

Discussion

In the present study we found that verapamil potentiated lipolysis due to Bt₂cAMP at sub-maximal concentrations (Fig. 1). However, vera-

Fig. 5. *Effect of α-adrenergic antagonists on lipolysis due to Bt₂cAMP*

Isolated adipocytes were incubated with 0.5 mM-Bt₂cAMP in the presence (open symbols) or absence (closed symbols) of 25 μM-verapamil in Ca²⁺-minus (10 μM-EGTA) medium for 60 min at 37°C with the indicated concentrations of yohimbine (□, ■), dibenamine (◇, ◆) or phenoxybenzamine (△, ▲). Results are means for triplicate determinations from three separate experiments.

pamil showed only an inhibitory effect on lipolysis due to adrenaline or IBMX (Figs. 3 and 4). Fain (1970) observed similar paradoxical phenomena with dihydroergotamine. Ward & Fain (1971) found that dihydroergotamine inhibited lipolysis due to adrenaline by interfering with stimulation of adenylate cyclase while it potentiates lipolysis due to Bt₂cAMP by the inhibition of cyclic AMP phosphodiesterase. Epstein *et al.* (1982) have shown that verapamil inhibited calmodulin-insensitive phosphodiesterase from rat heart and antagonized the stimulation of the calmodulin-sensitive enzyme from rat brain. Thus verapamil might at least in part exert these effects by a mechanism similar to that exerted by dihydroergotamine.

Since verapamil is lipophilic, it is possible that it distributes into membrane structures (Bandi, 1978) and perturbs them (Fairhurst *et al.*, 1980). The perturbation of membrane structures may cause increases of permeability of Bt₂cAMP, which is more lipophilic than cyclic AMP, and subsequent increases of intracellular Bt₂cAMP concentrations and potentiation of lipolysis.

Verapamil has been found to interact with α-adrenergic receptors. Verapamil prevented [³H]-yohimbine binding to α₂-adrenoreceptors in rat brain membranes (Frelin *et al.*, 1982). Blackmore *et al.* (1979) found that verapamil inhibited the binding of [³H]adrenaline to α-adrenergic receptors and inhibited the stimulatory effects of adrenaline and phenylephrine on glycogenolysis and

phosphatase phosphorylase activation in rat liver cells. Barnathan *et al.* (1982) reported that verapamil inhibited adrenaline-induced aggregation at the level of the platelet α -adrenergic receptors. Adipocyte-cell membranes have both α - and β -adrenergic receptors (Meisner & Carter, 1977). Lawrence & Larner (1977) found that the activation of α -adrenergic receptors leads to an increase in phosphorylase *a* activity and a decrease in glycogen synthetase I activity in rat adipocytes. Thus verapamil might potentiate lipolysis due to Bt_2cAMP at the level of the α -adrenergic receptors. However, this is not the case, since α -adrenergic antagonists did not block the effects of verapamil (Fig. 5).

Since verapamil potentiates lipolysis due to Bt_2cAMP even in the absence of extracellular Ca^{2+} , it does not exert its effect by blocking extracellular Ca^{2+} influx. However, this does not rule out the possibility that Ca^{2+} is concerned in the effects of verapamil. Smooth endoplasmic reticulum (Hales *et al.*, 1974) and mitochondria (Epping *et al.*, 1983) store Ca^{2+} . It has been suggested that lipolysis may be controlled by intracellular Ca^{2+} redistribution (Siddle & Hales, 1974; Gaion & Krishna, 1982). Thus verapamil might exert its effect by affecting intracellular Ca^{2+} regulation even without extracellular Ca^{2+} . However, the exact mechanism of the action of verapamil is unclear and further studies are required to elucidate it.

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