Histamine-Induced Depolarization and the Cyclic AMP–Protein Kinase A System in Isolated Guinea Pig Adipocytes

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ABSTRACT — The relationship between histamine (Hi)-induced depolarization and the cyclic AMP system in adipocytes was studied in guinea pigs, which seem to be more sensitive than rats to Hi. Hi caused a dose-dependent depolarization in guinea pig mesenterial and epididymal adipocytes with EC_{50} values of 1.69×10^{-7} M and 1.19×10^{-7} M, respectively. Guinea pig adipocytes were 280–750 times more sensitive than rat adipocytes to Hi. Isoproterenol, forskolin and 3-isobutyl-1-methylxanthine (IBMX) also caused a depolarization, and the slopes of the concentration response lines for these drugs were almost the same as that for Hi. Furthermore, pretreatment with these drugs resulted in a potentiation of Hi-induced depolarization at lower concentrations which are not effective when each drug is used alone. In addition, Hi-induced depolarization was inhibited by pretreatment with prostaglandin E_1 (PGE_1) and insulin dose-dependently. The content of cyclic AMP in adipocytes was increased by Hi (10^{-7} M) in association with a decrease in membrane potential. KT5720, a protein kinase A inhibitor, which provides no significant effect even at a concentration of 10^{-6} M, showed an antagonistic effect on Hi-induced depolarization.

Keywords: Histamine, Adipocytes, Depolarization, Cyclic AMP

We previously found that intravenous infusion of histamine (Hi) elicited an elevation of free fatty acid (FFA) levels in dog plasma. Furthermore, Hi-induced lipolysis took place through an activation of H_2-receptors; An H_2-agonist, 4-methylHi, exerted the same effect as Hi, and H_2-antagonists, cimetidine and ranitidine, blocked Hi-induced lipolysis (1). Cheng et al. (2) reported that the relative potency of various catecholamines on membrane depolarization corresponds closely with concentrations reported to stimulate lipolysis in adipocytes. The effects of Hi and related compounds on rat mesenterial adipocytes have also been investigated using electrophysiological techniques (3). However, a relatively high concentration of Hi is necessary to exert a significant effect in rat adipocytes (3). It is generally thought that guinea pigs are more sensitive to Hi than rats, although little work has been done concerning the effect of Hi on guinea pig adipocytes.

It is known that stimulation of H_2-receptors by Hi induces cyclic AMP formation in the brain, stomach and cardiac muscle in various species (4, 5). Also, we have reported that when rat adipocytes were exposed to dibutyryl cyclic AMP or intracellular application of cyclic AMP was carried out, a significant depolarization took place, although the relation between Hi-induced depolarization and the cyclic AMP system is not yet clearly understood (3). Therefore, the present study was performed to confirm whether or not cyclic AMP actually participates in Hi-induced depolarization.

MATERIALS AND METHODS

Measurement of membrane potential

Male Wistar rats weighing 400–500 g and male Hartley guinea pigs weighing 600–700 g were used. After decapitation, the mesenterial or epididymal fat pad was rapidly excised and cut into small pieces. The fat pad was stretched lightly and fixed with stainless steel needles on silicone rubber embedded in the test chamber. The volume of the test chamber was 2 ml, and the fat pad was superfused with Krebs Ringer bicarbonate (KRB) buffer gassed with 95% O_2 and 5% CO_2 at a constant rate of 3 ml/min. The remaining space of the test chamber was filled with the same gas to keep the

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pH in the chamber nearly 7.4, and the temperature was maintained at 32 ± 1°C. The intracellular membrane potential was measured by means of a glass microelectrode filled with 3 M KCl, and the electrical resistance was approximately 30 to 40 MΩ; tip potentials were less than 10 mV. Membrane potentials were amplified with an amplifier having an input impedance greater than 10^{12} Ω (Nihon Kohden, MEZ-8201) and were displayed on a dual-beam memory oscilloscope (Nihon Kohden, VC-10). Effective membrane resistance was calculated from the electrotonic potentials induced by applying the square current (10^{-9} A, 100 msec duration).

Solution and drugs

The solution consisting of: 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11.1 mM glucose (pH 7.4), was equilibrated with 95% O_2 and 5% CO_2 gas. The following drugs were used: Hi dihydrochloride (Wako), *l*-isoproterenol hydrochloride (Nikken), forskolin (Sigma), 3-isobutyl-1-methylxanthine (IBMX, Sigma), prostaglandin E_1 (PGE_1, Ono), insulin (Sigma) and KT5720 (Kyowa Hakko).

Measurement of cyclic AMP level

Epididymal fat pads were sliced and incubated in 5 ml of the KRB-albumin buffer containing 10 mg of collagenase. Incubation was carried out for 30 min at 37°C in an atmosphere of 95% O_2 and 5% CO_2. Thereafter, the isolated cells were collected as described by Rodbell (6). The fat cells (10^5 cells/ml) were preincubated for 10 min, and after the addition of Hi, incubation was continued. The reaction was terminated by an addition of 2 ml of 3% TCA. After centrifugation (1,000 × *g*, 30 min), the protein precipitate was removed, the samples were washed four times with 5 vol. of water-saturated ether, and then evaporated to dryness. The residue was dissolved in 0.1 ml of distilled water and cyclic AMP was determined by a radioimmunoassay kit (Yamasa).

Statistics

The data are expressed as the mean ± S.E.M. (n = 7–15 cells obtained from 3 different animals). Statistical analyses were performed by one-way analysis of variance, and the significance of the difference between the control and drug-treated groups was calculated by Dunnett's test. EC_{50} was calculated by the method of Litchfield and Wilcoxon.

RESULTS

Resting membrane potentials, effective resistance and cell sizes of adipocytes in rats and guinea pigs

As shown in Table 1, the resting membrane potentials of rats and guinea pigs in mesenterial and epididymal adipocytes ranged from −21.1 ± 0.6 to −23.4 ± 0.9 mV, and no significant difference between these 4 groups was detected. On the other hand, the effective membrane resistances of mesenterial and epididymal adipocytes in guinea pigs were significantly lower than those determined in the corresponding cells obtained from rats. There was no significant difference between the size of rat and guinea pig adipocytes.

Effect of Hi on resting membrane potentials of mesenterial and epididymal adipocytes in rats and guinea pigs

Figures 1 and 2 show the effect of Hi on membrane potentials in rats and guinea pigs. In rat mesenterial adipocytes, a significant depolarization was observed at concentrations higher than 5×10^{-6} M, whereas in

Table 1. Comparison of the resting membrane potentials, effective membrane resistances and cell sizes of adipocytes in rats and guinea pigs

Animal	Adipocyte	Resting membrane potential (mV)	Effective membrane resistance (MΩ)	Cell size (μm)
Rat	Mesenterial	−21.1 ± 0.6 (n = 200)	19.3 ± 0.8 (n = 200)	67.1 ± 2.5 (n = 50)
Rat	Epididymal	−22.4 ± 1.2 (n = 50)	19.2 ± 2.4 (n = 50)	76.5 ± 2.0 (n = 30)
Guinea pig	Mesenterial	−22.8 ± 0.8 (n = 50)	9.83 ± 0.9** (n = 50)	68.9 ± 1.8 (n = 30)
Guinea pig	Epididymal	−23.4 ± 0.9 (n = 50)	12.9 ± 0.9* (n = 50)	69.7 ± 2.6 (n = 30)

*, **: Significantly different from the effective membrane resistance of mesenterial and epididymal adipocytes of rats with $P < 0.05$ and $P < 0.01$, respectively.

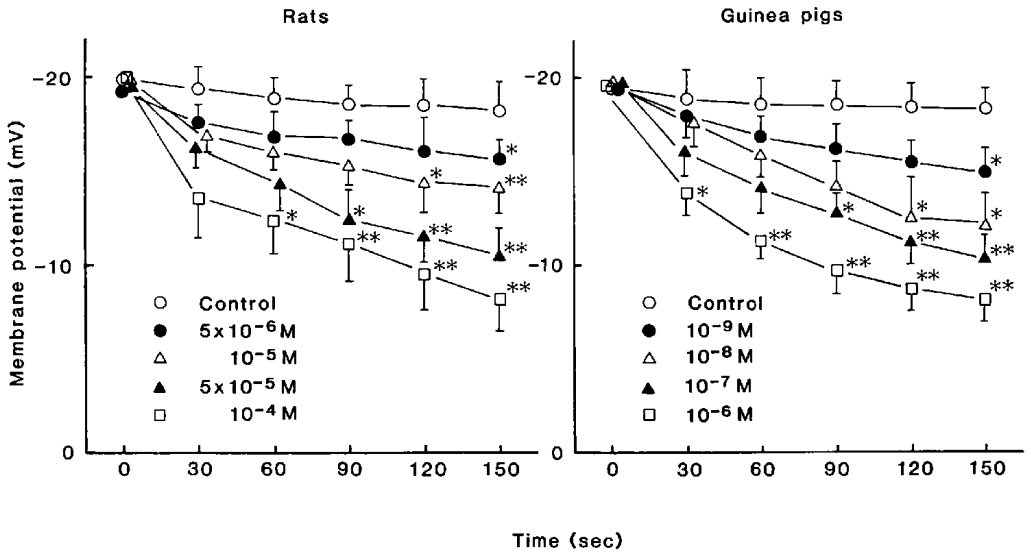

Fig. 1. Effect of histamine on membrane potentials of mesenterial adipocytes in rats and guinea pigs. *P < 0.05, **P < 0.01.

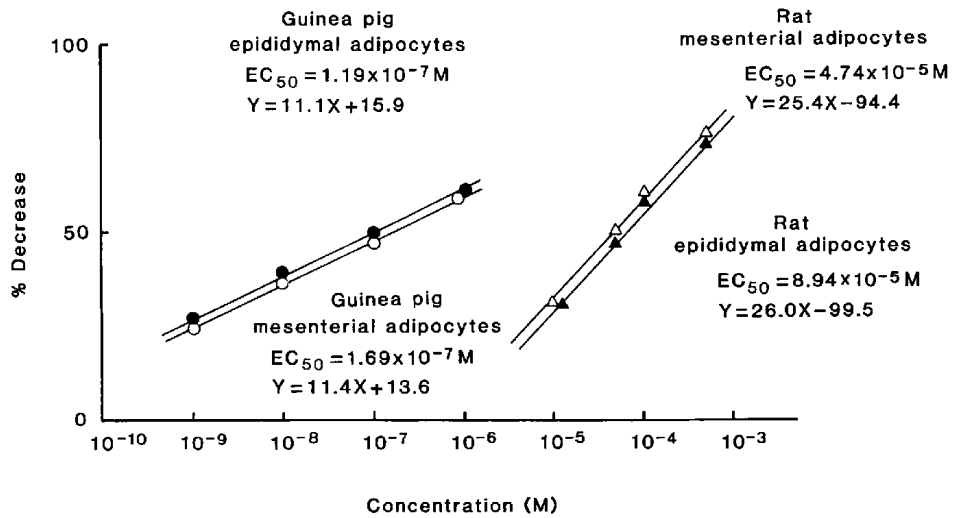

Fig. 2. Concentration-response lines of histamine in adipocytes of rats and guinea pigs in the determination of membrane potentials.

guinea pig adipocytes, a significant effect was detected even at a concentration of 10^{-9} M (Fig. 1). In mesenterial fat cells, EC_{50}s for Hi-induced depolarization in rats and guinea pigs were 4.74×10^{-5} M and 1.69×10^{-7} M, respectively, indicating that guinea pig cells are about 280 times more sensitive than rat cells to Hi. Similar results were also obtained for epididymal adipocytes (Fig. 2).

Effects of isoproterenol, forskolin and IBMX on resting membrane potentials in mesenterial adipocytes of guinea pigs

As shown in Table 2, isoproterenol elicited a concentration-related depolarization, and a significant effect was observed at concentrations higher than 10^{-7} M. Forskolin, an adenylate cyclase activator, and IBMX, a phosphodiesterase inhibitor, also caused concentration-dependent depolarizations. Significant depolarizations of forskolin and IBMX were observed at concentrations higher than 10^{-9} M and 10^{-8} M, respectively. Figure 3 shows concentration-response lines for Hi, isoproterenol, forskolin and IBMX. EC_{50}s for Hi, isoproterenol, forskolin and IBMX were 1.69×10^{-7} M, 1.61×10^{-6} M, 6.40×10^{-8} M and 1.45×10^{-7}

Table 2. Effects of isoproterenol, forskolin and IBMX on resting membrane potentials in mesenterial adipocytes of guinea pigs

Drugs	Concentration (M)	Membrane potential (mV ± S.E.M.) 0	30	60	90	120	150 sec
Control	–	21.2 ± 2.1	20.7 ± 2.3	20.4 ± 2.3	20.4 ± 2.4	20.1 ± 2.3	20.0 ± 2.4
Isp	10^{-8}	21.4 ± 1.2	18.8 ± 1.2	17.3 ± 1.4	16.8 ± 1.6	16.3 ± 1.7	15.7 ± 1.7
	10^{-7}	19.6 ± 1.0	17.6 ± 1.2	16.9 ± 1.9	15.4 ± 1.8	13.9 ± 2.0*	13.2 ± 2.2*
	10^{-6}	20.2 ± 1.3	17.4 ± 1.7	15.8 ± 1.7	13.6 ± 1.8**	12.2 ± 1.8**	10.8 ± 1.7**
	10^{-5}	20.2 ± 1.2	16.5 ± 1.7	14.0 ± 1.1**	10.6 ± 1.8**	9.2 ± 2.0**	7.7 ± 1.8**
Fors	10^{-10}	20.1 ± 1.0	18.9 ± 1.3	17.7 ± 1.3	17.0 ± 1.4	16.9 ± 1.5	16.5 ± 1.6
	10^{-9}	19.9 ± 1.1	17.9 ± 2.0	16.9 ± 2.0	15.4 ± 1.7	15.1 ± 1.6*	14.4 ± 1.6*
	10^{-8}	19.6 ± 1.3	15.4 ± 1.8	13.8 ± 0.9**	12.4 ± 1.1**	11.7 ± 1.4**	11.0 ± 1.4**
	10^{-7}	19.7 ± 0.9	14.1 ± 1.8*	12.0 ± 2.1**	9.1 ± 1.8**	8.9 ± 1.7**	9.0 ± 1.7**
	10^{-6}	20.1 ± 1.4	13.4 ± 1.8**	11.4 ± 2.6**	8.3 ± 2.8**	8.1 ± 2.9**	7.7 ± 2.9**
IBMX	10^{-9}	20.1 ± 1.3	18.5 ± 1.5	17.4 ± 1.6	17.1 ± 1.8	16.6 ± 1.7	15.6 ± 1.5
	10^{-8}	20.0 ± 0.7	18.3 ± 0.9	17.2 ± 1.0	15.6 ± 1.1**	14.3 ± 1.2**	13.4 ± 1.5**
	10^{-7}	20.0 ± 1.1	17.4 ± 1.2	15.7 ± 1.2*	14.0 ± 1.1**	12.1 ± 1.1**	10.4 ± 1.2**
	10^{-6}	19.9 ± 1.4	16.7 ± 1.5	14.9 ± 1.0**	12.3 ± 1.2**	9.5 ± 1.3**	8.1 ± 1.4**

*, **: Significantly different from the control group with $P < 0.05$ and $P < 0.01$, respectively. Isp: Isoproterenol, Fors: Forskolin, IBMX: 3-Isobutyl-1-methylxanthine. N = 7–12.

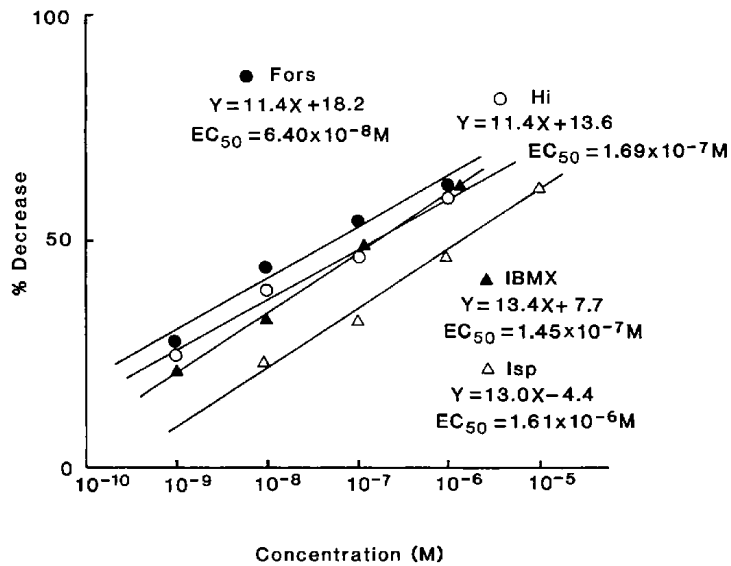

Fig. 3. Concentration-response lines for the decrease of resting membrane potentials in mesenterial adipocytes of guinea pigs due to some lipolytic agents. Hi: Histamine, Isp: Isoproterenol, Fors: forskolin, IBMX: 3-Isobutyl-1-methylxanthine.

M, respectively. The effect of forskolin was slightly more potent than that of Hi, while IBMX was almost the same as Hi. The effect of isoproterenol was slightly less potent than that of Hi. There was no significant difference between the slopes of the concentration-response lines of these four agents.

Effect of isoproterenol on the decrease of membrane potentials induced by Hi in mesenterial adipocytes of guinea pigs

When isoproterenol alone was added at 10^{-8} M to the adipocytes, no change in membrane potential was observed. However, when they were similarly pretreated with isoproterenol, Hi (10^{-10} M)-induced depolarization was significantly increased as shown in Fig. 4A; and in the presence of isoproterenol (10^{-8} M), the concentration-response line for Hi shifted in parallel to the left (Hi alone: Y = 11.4X + 13.6; Hi + isoproterenol: Y = 10.8X + 26.8, Fig 4B).

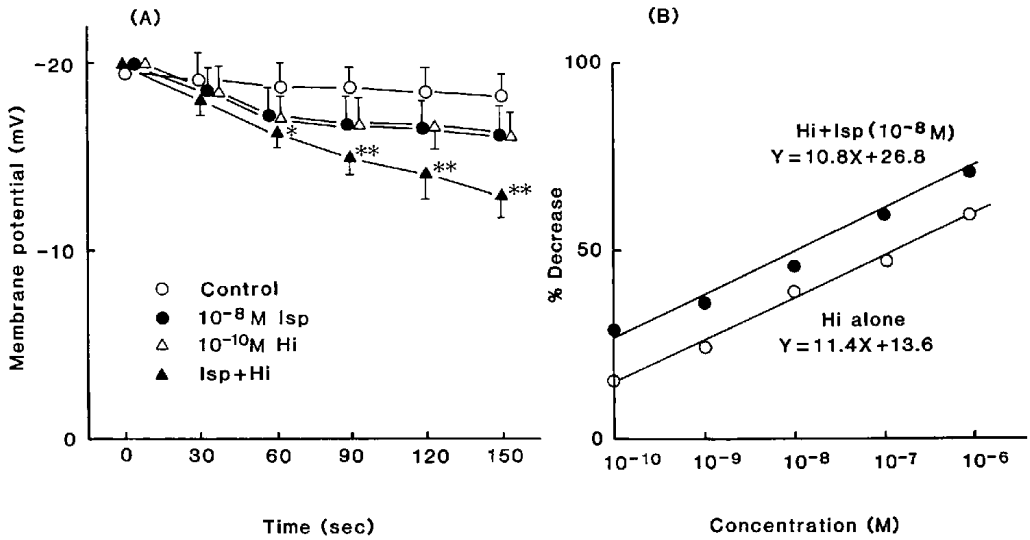

Fig. 4. Effect of isoproterenol on the decrease of membrane potential induced by histamine in mesenterial adipocytes of guinea pig. Hi: Histamine, Isp: Isoproterenol. *P < 0.05, **P < 0.01.

Effects of forskolin and IBMX on Hi-induced depolarization in mesenterial adipocytes

As shown in Table 3, forskolin (10^{-10} M) alone induced no significant changes in membrane potential, while the Hi (10^{-10} M)-induced depolarization was augmented in the presence of forskolin (10^{-10} M). Similar potentiation in membrane depolarization was observed with the combination of IBMX (10^{-9} M) and Hi (10^{-10} M).

Effects of PGE_1 and insulin on Hi-induced depolarization in mesenterial adipocytes

Pretreatment with PGE_1 at 10^{-7} M and 10^{-6} M exerted a significant antagonizing effect on Hi-induced depolarization (Table 4). Insulin also antagonized the Hi-induced depolarization at concentrations of 1 and 10 mU/ml. When PGE_1 and insulin were applied individually, no significant changes in membrane potential were induced even at concentrations of 10^{-6} M and 10 mU/ml, respectively (data not shown).

Effect of Hi (10^{-7} M) on resting membrane potentials and cyclic AMP levels in epididymal adipocytes of guinea pigs

As shown in Fig. 5, significant depolarization was brought about at 90 sec after addition of Hi. In contrast, the cyclic AMP level was increased in relation to membrane depolarization of the adipocytes. A significant increase in cyclic AMP was detected at 60 sec after stimulation by Hi.

Inhibitory effect of KT5720 on the depolarization induced by Hi in mesenterial adipocytes in guinea pigs

KT5720, a specific protein kinase A inhibitor (7),

Table 3. Effects of forskolin and IBMX on histamine-induced depolarization in mesenterial adipocytes of guinea pigs

Drugs	Concentration (M)	Depolarization (mV ± S.E.M.) 30	60	90	120	150 sec
Control	—	0.8 ± 0.2	1.2 ± 0.3	1.6 ± 0.4	2.1 ± 0.4	2.3 ± 0.3
Hi	10^{-10}	1.0 ± 0.5	1.8 ± 0.5	2.3 ± 0.4	2.3 ± 0.4	2.9 ± 0.4
Fors	10^{-10}	1.3 ± 0.5	2.3 ± 0.7	2.8 ± 0.9	2.9 ± 1.1	3.1 ± 1.1
Hi + Fors		2.4 ± 0.7	4.8 ± 1.2*	6.0 ± 1.5*	6.8 ± 1.6**	7.6 ± 1.7**
IBMX	10^{-9}	1.6 ± 0.6	2.3 ± 0.6	2.8 ± 0.8	3.2 ± 0.8	4.1 ± 0.9
Hi + IBMX		2.6 ± 0.5	3.7 ± 0.6	5.3 ± 0.7**	6.6 ± 0.6**	6.9 ± 0.6**

*, **: Significantly different from the histamine-treated group with P < 0.05 and P < 0.01, respectively. Hi: Histamine, Fors: Forskolin, IBMX: 3-Isobutyl-1-methylxanthine. N = 8 - 10.

Table 4. Effects of PGE_1 and insulin on histamine-induced depolarization in mesenterial adipocytes of guinea pigs

Drugs	Concentration (M)	Depolarization (mV ± S.E.M.) 30	60	90	120	150 sec
Hi	10^{-6} M	2.6 ± 0.3	4.6 ± 0.8	5.8 ± 1.0	7.2 ± 1.1	8.9 ± 1.3
Hi + PGE_1	10^{-8} M	2.5 ± 0.9	3.3 ± 1.1	5.3 ± 1.6	6.8 ± 1.5	8.2 ± 1.9
	10^{-7} M	2.3 ± 0.4	3.1 ± 1.1	4.0 ± 1.1	4.4 ± 1.0*	4.8 ± 0.9*
	10^{-6} M	1.8 ± 0.5	2.3 ± 0.4*	2.8 ± 0.7*	3.1 ± 0.9*	3.5 ± 1.1**
Hi + Ins	0.1 mU/ml	2.3 ± 0.5	2.7 ± 0.8	4.1 ± 1.0	5.3 ± 1.2	6.8 ± 1.2
	1 mU/ml	2.0 ± 0.4	2.2 ± 1.0	3.4 ± 1.0	3.4 ± 0.9*	4.7 ± 1.3*
	10 mU/ml	0.3 ± 0.2**	1.5 ± 0.5**	2.8 ± 0.7**	2.9 ± 0.7**	3.4 ± 0.8**

*, **: Significantly different from the histamine-treated control group with $P < 0.05$ and $P < 0.01$, respectively. Hi: Histamine, PGE_1: Prostaglandin E_1. Ins: Insulin. N = 7–10.

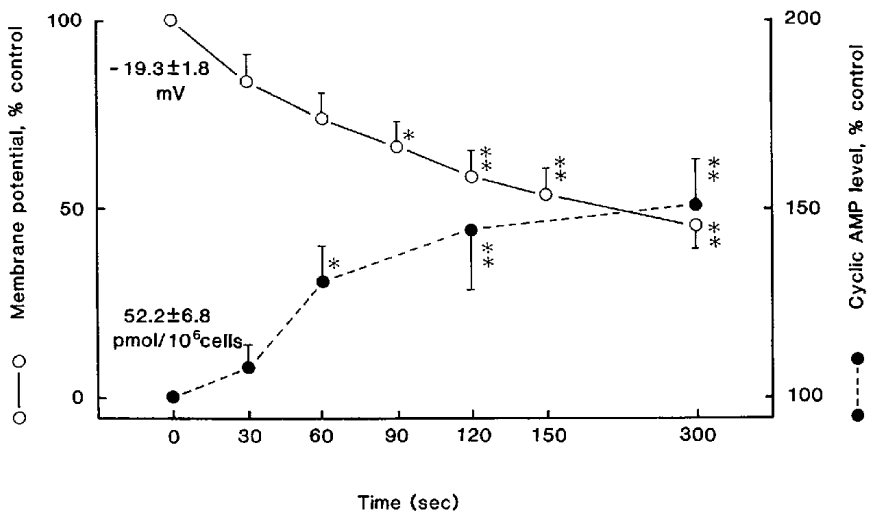

Fig. 5. Effect of histamine (10^{-7} M) on resting membrane potentials and cyclic AMP levels in epididymal adipocytes of guinea pigs. *$P < 0.05$, **$P < 0.01$.

caused no significant depolarization even at a concentration of 10^{-6} M. Pretreatment with KT5720 resulted in a significant and dose-related inhibition of Hi-induced depolarization at concentrations higher than 10^{-8} M (Fig. 6).

DISCUSSION

In the present experiment, it was found that guinea pig cells are more sensitive than rat cells to Hi. As an indication of this, the EC_{50} of Hi-induced depolarization in rat mesenterial adipocytes was 4.74×10^{-5} M, whereas in guinea pig adipocytes, it was 1.69×10^{-7} M. This relation was consistent with those of the epididymal adipocytes. Furthermore, in the case of isoproterenol, guinea pigs were also more sensitive than rats. Previously, we reported that isoproterenol caused a depolarization in rat mesenterial adipocytes with an EC_{50} of 7.78×10^{-5} M (3), whereas in the present study, the EC_{50} of isoproterenol was 1.61×10^{-6} M in the adipocytes obtained from guinea pig mesentery. These findings seem to suggest that guinea pig adipocytes may be more sensitive than rat adipocytes. This finding may be related to the fact that the effective membrane resistance of adipocytes is much lower in guinea pigs than in rats.

Hi-induced depolarization was significantly potentiated by pretreatment with isoproterenol, and the linear concentration-response curve of Hi was shifted in parallel to the left. In addition, the slope of the concentration-response curve was almost the same for both drugs. These results seem to indicate that a common mechanism may exist between Hi- and isoproterenol-induced depolarization. Cheng et al. (2) reported that dibutyl cyclic AMP induced membrane depolarization, and this effect was enhanced by theophylline. Since the

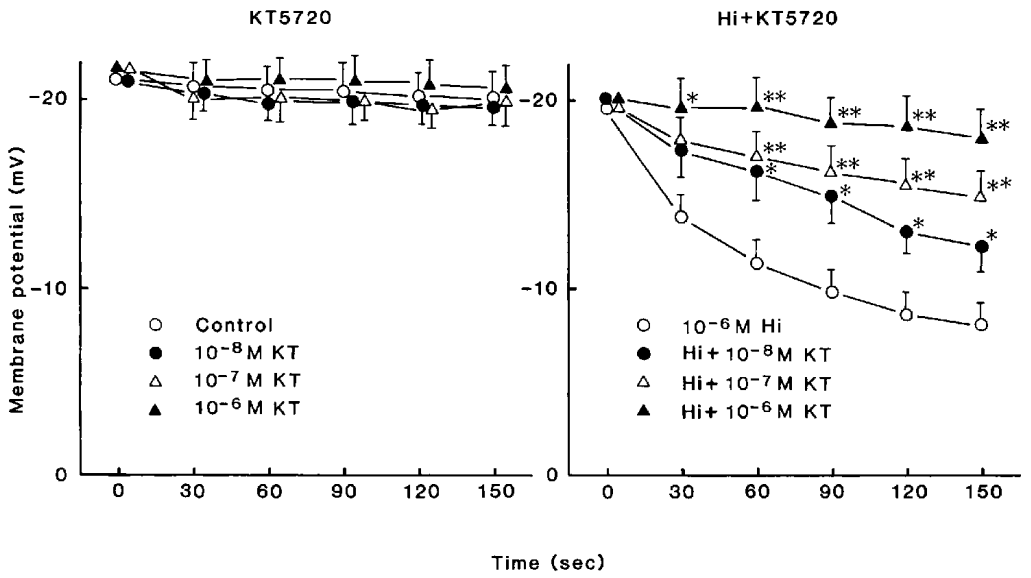

Fig. 6. Effect of KT5720 on the decrease of resting membrane potentials induced by histamine in mesenterial adipocytes of guinea pigs. KT: KT5720. *P < 0.05, **P < 0.01.

discovery of Sutherland et al. (8), it is generally recognized that catecholamines stimulate adenylate cyclase activity, and this process leads to the formation of cyclic AMP. Therefore, we tested the effect of forskolin, which is a potent and unique activator of adenylate cyclase (9, 10). As shown in Table 2 and Fig. 3, forskolin induces a depolarization almost the same as or somewhat more potent than that of Hi, and the slope of the concentration-response curve of forskolin is entirely in agreement with that of Hi. Litosch et al. (11) reported that forskolin stimulated lipolysis in intact rat adipocytes with an EC_{50} of approximately 5×10^{-6} M as assessed by glycerol release. This finding is almost the same as the present result. In addition, as shown in Table 3, Hi-induced depolarization was significantly augmented by pretreatment with forskolin. Furthermore, in the presence of PGE_1, which is known to be one of the most potent inhibitor of adenylate cyclase in hamster adipocytes (12), depolarization induced by Hi was markedly inhibited (Table 4). There have been some reports that Hi stimulates adenylate cyclase of myocardial tissues in guinea pigs (13) and adipocytes in dogs (14). It is therefore very likely that Hi-induced depolarization of adipocytes is intimately related to adenylate cyclase.

It is known that IBMX is a potent inhibitor of phosphodiesterase and that it causes an increase of cyclic AMP contents in the guinea pig papillary muscle (15). In the present study, IBMX caused a depolarization and its pretreatment potentiated Hi-induced depolarization as shown in Table 3. On the other hand, insulin has been reported to control intracellular cyclic AMP concentration in hepatocytes by activating the specific cyclic AMP phosphodiesterase (16). Therefore, insulin was used as a representative phosphodiesterase activator. As shown in Table 4, insulin exerted an antagonistic effect on Hi-induced depolarization. This finding is probably in accord with the observation made by Cheng et al. (2) that insulin antagonized norepinephrine-induced reduction of the membrane potential of brown adipocytes in rats.

The observation that Hi increased cyclic AMP levels in guinea pig adipocytes in direct proportion to a decrease of membrane potential, may indicate that Hi-induced depolarization probably takes place in association with an increase in cyclic AMP. Grund et al. (17) reported that Hi increased cyclic AMP levels in a dose-dependent fashion in isolated dog fat cells, and that a rapid increase in cyclic AMP levels precedes an elevation of free fatty acid. These results suggest that elevation of cyclic AMP level is necessary to trigger the lipolysis. Furthermore, it is known that lipolytic agents such as epinephrine and glucagon stimulate lipolysis by increasing the cellular cyclic AMP concentration, resulting in activation of protein kinase A (18). Honnor et al. (19) found that a good relationship exists between the activation state of protein kinase A and lipolysis in rat adipocytes. Recently, Kase et al. (7) found that the compound KT5720 selectively inhibits protein kinase A. Accordingly, the effect of KT5720 on Hi-induced depolarization was investigated, and it was found that KT5720 exerted a marked inhibitory effect on Hi-in-

duced depolarization. Thus, it can be assumed that Hi-induced depolarization is intimately related in some way to protein kinase A.

Thus, it can be concluded that guinea pigs seem to be more sensitive to Hi than rats in membrane depolarization of adipocytes, and Hi-induced depolarization may be intimately related to cyclic AMP – protein kinase A system.

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