ABSTRACT—Effects of ions on histamine (Hi)-induced depolarization were studied in guinea pig adipocytes. Depolarization induced by Hi in guinea pig adipocytes was decreased by removal of K+ from the medium or pretreatment with ouabain at concentrations that showed no significant effect themselves. The decrease in membrane potentials induced by Hi was also abolished potently by replacement of Na+ by choline or pretreatment with tetrodotoxin at a concentration that caused no significant action alone. Pretreatment with monensin at a concentration lower than that eliciting the action resulted in a potentiation of Hi-induced depolarization. The depolarization induced by Hi was not affected by the presence of Ca2+ in the medium or pretreatment of the cells by diltiazem.

Keywords: Histamine, Depolarization, Tetrodotoxin, Monensin, Adipocyte (guinea pig)
Statistics

The data are expressed as means ± S.E.M. (N = 10–15, cells obtained from 5 different animals). Statistical analysis was 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 (6).

RESULTS

Effect of Hi on membrane potentials of mesenterial adipocytes of guinea pigs

Hi at concentrations higher than 10^-9 M caused concentration-related depolarizations in mesenterial adipocytes as shown in Fig. 1. After addition of Hi at a concentration of 10^-9 M, membrane potentials decreased gradually and reached a significant level at 150 sec. At a concentration of 10^-6 M, Hi produced potent depolarization, and a significant change was noted at 30 sec after addition of the drug (Fig. 1).

Influence of K+ removal from the medium on Hi-induced depolarization

Removal of K+ from the medium resulted in a gradual depolarization, and a significant effect was observed 180 sec after exposure to K+ -free medium (Fig. 2). The depolarization induced by Hi (10^-6 M and 10^-5 M) was inhibited significantly at 150 sec after the addition of Hi in the K+ -free medium (Table 1).

Table 1. Influence of K+ removal on histamine-induced depolarization of guinea pig adipocytes

| Medium                  | Depolarization (mV ± S.E.M.) | N  |
|-------------------------|------------------------------|----|
| Normal medium           |                              |    |
| 10^-6 M Histamine       | 8.6±1.2                      | 14 |
| 10^-3 M Histamine       | 12.3±1.9                     | 12 |
| K+ free medium          |                              |    |
| 10^-6 M Histamine       | 5.2±1.2*                     | 13 |
| 10^-3 M Histamine       | 6.7±1.2*                     | 11 |

*: Significantly different from the histamine-treated group in normal medium with P < 0.05.

Effect of ouabain on Hi-induced depolarization

Ouabain at a concentration of 10^-7 M induced no significant changes in membrane potential, although when the adipocytes were pretreated by ouabain at the same concentration, Hi-induced depolarization was prevented. Inhibition became apparent as the time passed, and it became significant from 90 sec after the addition of Hi at concentrations of 10^-6 M and 10^-5 M (Fig. 3).

Effect of replacement of Na+ by choline on Hi-induced depolarization

Replacement of Na+ by choline depolarized the cells, and a significant effect was observed at 180 sec after ex-
Exposure to Na⁺-free medium (Fig. 4). The decrease in membrane potentials induced by Hi at concentrations of 10⁻⁶ M and 10⁻⁵ M was significantly inhibited by replacement of Na⁺ by choline 150 sec after the addition of Hi (Table 2).

**Effect of tetrodotoxin on Hi-induced depolarization**

No significant changes in membrane potentials were induced by tetrodotoxin at 5 x 10⁻⁹ M. Also, at the same concentrations, tetrodotoxin was effective in inhibiting Hi-induced depolarization (Fig. 5).

**Effect of monensin on Hi-induced depolarization**

Pretreatment with monensin at 10⁻⁸ M resulted in a significant potentiation of Hi (10⁻⁹ M)-induced depolarization (Fig. 6). No changes in membrane potential was elicited by monensin at concentrations lower than 10⁻⁶ M.

### Table 2. Effect of replacement of Na⁺ by choline on histamine-induced depolarization of guinea pig adipocytes

| Medium                  | Depolarization (mV ± S.E.M.) | N  |
|-------------------------|------------------------------|----|
| Normal medium           |                              |    |
| 10⁻⁶ M Histamine        | 8.6±1.2                      | 14 |
| 10⁻⁵ M Histamine        | 12.3±1.9                     | 12 |
| Replacement of Na⁺ by choline |                      |    |
| 10⁻⁴ M Histamine        | 3.2±0.8**                    | 13 |
| 10⁻³ M Histamine        | 3.9±0.5**                    | 11 |

**: Significantly different from the histamine-treated group in normal medium with P<0.01.

**Fig. 3.** Effect of ouabain on histamine (Hi)-induced depolarization of guinea pig adipocytes. ○: 10⁻⁶ M Hi, ●: 10⁻⁵ M Hi, △: 10⁻⁶ M Hi+ouabain, ▲: 10⁻⁵ M Hi+ouabain. *:*: Significantly different from the histamine-treated groups with P<0.05 and P<0.01, respectively.

**Fig. 4.** Effect of replacement of Na⁺ by choline on membrane potentials of guinea pig adipocytes. The preparation was superfused during the time indicated by the arrows. ○: control, ●: Na⁺- free medium. *:*: Significantly different from the control group with P<0.05.

**Fig. 5.** Effect of tetrodotoxin on histamine (Hi)-induced depolarization of guinea pig adipocytes. ○: 10⁻⁶ M Hi, ●: 10⁻⁵ M Hi, △: 10⁻⁶ M Hi+tetrodotoxin, ▲: 10⁻⁵ M Hi+tetrodotoxin. *:*: Significantly different from the histamine-treated groups with P<0.05 and P<0.01, respectively.
Effect of removing Ca\(^{2+}\) from the medium on Hi-induced depolarization

Removal of Ca\(^{2+}\) from the medium exerted no significant effect on membrane potentials. Although the depolarization induced by Hi was somewhat low in Ca\(^{2+}\)-free medium compared with that determined in normal medium, no significant difference was observed after the addition of Hi (Table 3).

Effect of diltiazem on Hi-induced depolarization

At 10\(^{-8}\) M, diltiazem had almost no effect, inducing negligible changes in membrane potentials. In the depolarization induced by Hi, there was no significant difference between the group pretreated with diltiazem and that not given the pretreatment (Fig. 7).

**Table 3. Influence of Ca\(^{2+}\) removal from the medium on histamine-induced depolarization of guinea pig adipocytes**

| Medium                | Depolarization (mV ± S.E.M.) | N |
|-----------------------|------------------------------|---|
| Normal medium         |                              |   |
| 10\(^{-6}\) M Histamine | 8.6 ± 1.2                    | 14 |
| 10\(^{-7}\) M Histamine | 12.3 ± 1.9                   | 12 |
| Ca\(^{2+}\)-free medium |                            |   |
| 10\(^{-6}\) M Histamine | 7.5 ± 1.6                    | 13 |
| 10\(^{-3}\) M Histamine | 10.4 ± 1.6                   | 11 |

DISCUSSION

In the present study, it was found that Hi-induced depolarization was significantly inhibited in the K\(^+\)-free medium. A similar result was described by Williams and Matthews (7) in the norepinephrine-induced depolarization of rat brown adipocytes. In addition, it was also found that pretreatment with ouabain caused a significant inhibition of Hi- and isoproterenol-induced depolarization even in guinea pigs. Although the detailed mechanism remains unsolved, it is almost certain that if the normal functioning of the Na\(^+,K\(^+\)-pump was impaired, no depolarization could be produced by Hi.

It is well recognized that a decrease in [K\(^+\)]\(_i\) or an increase in [Na\(^+\)]\(_i\), resulted in gradual depolarizations of the cells. We also confirmed this finding in the present study; as shown in Fig. 2, membrane depolarizations takes place rather gradually in the K\(^+\)-free medium, and the extent of depolarization was also relatively weak in the K\(^-\)-free medium. On the contrary, as shown in Fig. 1, abrupt depolarizations were consistently observed after the addition of Hi. For instance, the membrane potentials dropped to half of the control level within 120 sec when Hi was added at a concentration of 10\(^{-7}\) M. It seems unlikely that a decrease in the total [K\(^+\)]\(_i\) takes place rapidly enough to produce such a pronounced change. As a possible explanation, a large increase in the permeability to Na\(^+\) can be assumed. Actually as shown in the present
study, removal of Na\(^+\) from the medium or addition of tetrodotoxin potently inhibited the Hi-induced depolarization compared with that seen in the K\(^-\)-free medium or addition of ouabain. Therefore, it is suggested that Hi-induced depolarization would be intimately related to the influx of Na\(^+\) into the cells. This assumption is probably supported by the fact that the monensin which is known as a lipophilic Na ionophore significantly prompted depolarizations.

It was found that removal of Ca\(^{2+}\) from the medium caused no significant changes in membrane potentials in adipocytes. A representative Ca-entry blocker, diltiazem (10\(^{-8}\) M), also induced no depolarizations of mesenterial adipocytes, although at high concentrations (10\(^{-6}\) M and 10\(^{-7}\) M), diltiazem caused a significant depolarization. Although the effect was somewhat less than that seen in normal medium, depolarizations induced by Hi (10\(^{-6}\) M) still occurred in the presence of diltiazem at the concentration of 10\(^{-8}\) M. This finding probably has a meaning similar to the results on norepinephrine reported by Williams and Matthews (7). They also found that in Ca\(^{2+}\)-free medium, norepinephrine induced a depolarization comparable to that seen in control medium. Furthermore, Mosinger and Kujalová (8) also described that in the ACTH-induced lipolysis, the presence of Ca\(^{2+}\) was inevitable, but no such requirement existed in epinephrine-induced lipolysis.

From these findings, it is reasonable to assume that Ca\(^{2+}\) may not be necessary for Hi-induced depolarization as in the case of norepinephrine.

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