EFFECTS OF 2-DEOXYGLUCOSE, PHLORIZIN, INSULIN AND OUABAIN ON GLUCOSE-DEPENDENT ANAPHYLACTIC HISTAMINE RELEASE FROM GUINEA-PIG LUNG

HIDEMASA YAMASAKI AND KOITI ENDO
Department of Pharmacology, Okayama University Medical School, Okayama

Received for publication July 27, 1964

The release of histamine in anaphylaxis has been shown to be blocked by anoxia and various enzyme inhibitors considered to interfere with metabolic energy-yielding processes (1-5). Several recent works (5-8) have indicated that glycolysis constitutes one of the sources of energy, at least under anoxic conditions, from the fact that presence of glucose prevents blocking actions both of anoxia and of inhibitors which interfere the oxidative metabolism. In our previous paper (3) reporting the enhancing effect of succinate on aerobic histamine release by antigen from sensitized guinea-pig lung tissue, a slight but similar effect of glucose was noted. This glucose effect was confirmed by Diamant (9, 10) not only in guinea-pig but in rat lung, while in the latter species succinate had no effect. Despite these discrepancies, some of energy required for the processes seems, even under aerobic conditions, to be delivered via glycolysis in both species. Therefore, it is interesting to investigate the influences on the glucose effect of substances which may interfere the metabolism of glucose or its transport across the cell membrane, under aerobic and anaerobic conditions. 2-Deoxyglucose, phlorizin, insulin and ouabain were chosen for the present study as such substances. This paper also present observations on the comparative effects of succinate and glucose on the anaphylactic release of histamine, along with the oxygen uptake, from the minced lung tissues of guinea pig and rat.

MATERIALS AND METHODS

Guinea pigs (either sex, 250-300 g) and rats (either sex, about 150 g) were sensitized by injection of crystallized egg albumin. Guinea pigs were injected subcutaneously with 0.1 g and intraperitoneally 0.1 g of egg albumin (1 ml, 10% solution). Rats were injected subcutaneously with 0.1 g of egg albumin, together with 1.3 ml of H. pertussis vaccine (1.5×10⁹ bacilli per ml), followed 3 days later by another subcutaneous injection of 0.1 g of egg albumin. The lungs of sensitized guinea pigs and rats were submitted to the experiments 3-5 weeks after the first sensitizing dose. The excised lungs were rinsed with saline solution and cut into fine pieces of 1.5×1 mm size. These particles were suspended in 50 ml of Krebs-Ringer solution [NaCl
128 mm, KCl 5.13 mM, CaCl₂·2H₂O 2.75 mM, MgSO₄·7H₂O 1.28 mM and phosphate buffer pH 7.4 (Na₂HPO₄ 100 mM and HCl 20 mM) 10% v/v] at 37°C for 20 minutes to remove spontaneously releasable histamine; then collected on a filter paper wetted with Krebs-Ringer solution and divided into masses of 200 mg each using Bang’s torsion balance.

In the main chamber of two armed Warburg flask 2.1 ml of Krebs-Ringer solution containing 200 mg of minced lung tissue were placed. One of the side arms contained 0.3 ml of egg albumin (final concentration, 0.1%) and the other contained 0.3 ml of Krebs-Ringer solution or the same solution dissolving substrates, sodium succinate or glucose. A piece of filter paper was placed in the inset and soaked with 0.2 ml of 20% potassium hydroxide solution. Into the main chamber 0.3 ml of metabolic inhibitors or other substances to be tested (2-deoxyglucose, phlorizin, insulin or ouabain) was added and then the flask was connected to the manometer. Air inside the flask was replaced with oxygen, and after 15 minute shaking substrates in one side arm and antigen (egg albumin) in the other were successively tipped into the main chamber to allow antigen-antibody reaction to proceed. Uptake of oxygen was measured for 30 minutes and computed in mm³/mg dry weight/hour (Qₒ₂). At the end of this shaking the disconnected flask was cooled immediately in ice water, and histamine of the supernatant and of tissue particles was assayed on atropinized guinea-pig ileum in a Tyrode bath by the method previously described (3, 11). Histamine release was computed in % of total histamine content. In random samples mepyramine completely inhibited the contractions of the ileum. All the substances contained in the samples (in the concentration used) did not affect the assay of histamine. In anaerobic experiments, air inside the flask was replaced with nitrogen gas. Metabolic inhibitors or other substances, when tested, were present in the main chamber being left in contact with tissue fragments under air for 15 minutes before nitrogenation. The pH of the test systems was maintained at around 7.4.

2-Deoxy-D-glucose (A grade) was obtained from California Corporation for Biochemical Research, phlorizin from Mann Research Laboratories, and ouabain (g-strophanthin, cryst.) and D-glucose from E. Merck AG. Ordinary insulin (Insulin Injection, J.P.) and other chemicals used were obtained from standard commercial sources.

RESULTS

1) Effects of glucose and succinate on histamine release and oxygen uptake in guinea-pig and rat lung tissues in in vitro anaphylaxis

As shown in Fig. 1, antigen-induced histamine release from guinea-pig lung tissue was increased more markedly by sodium succinate (10 mM) than by glucose (5.6 mM) under oxygen gas phase. The increase effected by succinate plus glucose was greater than that produced by succinate alone. Histamine release from rat lung tissue induced by antigen was not changed by succinate, while glucose produced an increase much larger than did in the case of guinea-pig lung tissue. Coexistence of succinate did not influence this effect of glucose alone.
In both species, the oxygen uptake was evidently enhanced in the presence of succinate, whereas no significant increase was produced by glucose. When both substrates were present, there was no further increase in oxygen uptake as compared to that in the presence of succinate alone. Identical experiments for the glucose effects on histamine release and oxygen uptake in guinea-pig lung tissue are reiterated also in Tables 1, 2 and 4.

**Table 1.** Inhibitory effect of 2-deoxyglucose (5.6 mM) on anaphylactic histamine release from guinea-pig lung tissue in presence or absence of glucose.

<table>
<thead>
<tr>
<th>Gas phase</th>
<th>Glucose (mM)</th>
<th>2-Deoxyglucose (mM)</th>
<th>O₂-uptake (Q₂)</th>
<th>Histamine release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>0</td>
<td>0</td>
<td>5.5 ± 0.30</td>
<td>15.8 ± 1.39</td>
</tr>
<tr>
<td>(4 expts.)</td>
<td>0</td>
<td>5.6</td>
<td>4.9 ± 0.42</td>
<td>5.7 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>5.6</td>
<td>0</td>
<td>6.1 ± 0.05</td>
<td>22.2 ± 1.60</td>
</tr>
<tr>
<td></td>
<td>5.6</td>
<td>5.6</td>
<td>5.9 ± 0.21</td>
<td>11.4 ± 1.57</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0</td>
<td>0</td>
<td>1.7 ± 0.30</td>
<td>1.8 ± 0.30</td>
</tr>
<tr>
<td>(4 expts.)</td>
<td>0</td>
<td>5.6</td>
<td>16.9 ± 0.56</td>
<td>16.9 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>5.6</td>
<td>0</td>
<td>1.7 ± 0.29</td>
<td>1.7 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>5.6</td>
<td>5.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In this and subsequent tables mean histamine release (as %) and Q₂, and standard error of the mean shown. P relates to the two immediately overlying values.
2) **Inhibitory effect of 2-deoxyglucose on the anaphylactic histamine release from guinea-pig lung tissue**

The results are shown in Table 1. Under oxygen gas phase, by the presence of glucose 5.6 mM anaphylactic histamine release again showed some increase accompanied with a slight increase of oxygen uptake. After the pretreatment with 2-deoxyglucose (5.6 mM) these histamine releases, in the presence and absence of glucose, were equally reduced by half or more the values of untreated controls. Under nitrogen, when the medium was devoid of glucose histamine release did not occur, i.e. depressed to the degree of spontaneous release; and this anoxic inhibition of histamine release was largely overcome by the presence of glucose 5.6 mM in the medium as already reported (5-8). This glucose-dependent histamine release was completely blocked by the pretreatment with the same molar concentration of 2-deoxyglucose. The oxygen uptake was practically not affected by the used concentration of this compound.

3) **Effects of phlorizin and insulin on the anaphylactic histamine release from guinea-pig lung tissue**

Under oxygen, phlorizin (1.0 mM) showed no significant influence on both histamine release and oxygen uptake in the in vitro anaphylaxis whether glucose (5.6 mM) was present or not. But, under nitrogen the histamine release produced by antigen in the glucose-containing medium was reduced to about 60% of untreated control (Table 2). Fig. 2 illustrates the inhibitory effect of 1.0 mM of phlorizin on the anoxic histamine release in the presence of various concentrations of glucose in the medium. From this figure it may be seen that the inhibition by phlorizin is relatively marked at the lower concentration of glucose where the glucose effect is rather insufficient.

Insulin alone had no effect on the histamine release. When present 15 minutes before the addition of 5.6 mM of glucose, 1.0 unit/ml of insulin did not show any influence on the glucose-effected histamine release. But, when the glucose concentration was increased to 22.4 mM (fourfold), remarkable enhancing effect was achieved even by a much lower concentration of insulin (0.1 unit/ml) (Fig. 3).

Sensitized lung tissue was incubated in the presence of 0.1 unit/ml of insulin for 30 minutes before the phlorizin (1.0 mM)-pretreatment for 15 minutes, followed by nitrogenation, glucose addition, then introduction of antigen. As shown in Table 3,
insulin clearly reversed the inhibitory effect of phlorizin on the glucose-dependent histamine release (Expt. No. 2). This effect of insulin was, however, not observed when the incubation time of insulin was shorter, e.g. 15 minutes (Expt. No. 1).

TABLE 3. Reversal by insulin of phlorizin-induced inhibition of anaphylactic histamine release from guinea-pig lung tissue in presence of glucose (5.6 mM) under nitrogen.

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Phlorizin (mM)</th>
<th>Insulin (unit/ml)</th>
<th>Histamine release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.* (4 expts.)</td>
<td>0</td>
<td>0</td>
<td>14.6±0.98</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td></td>
<td>9.3±0.83</td>
</tr>
<tr>
<td>1.0</td>
<td>0.1</td>
<td></td>
<td>9.7±1.31</td>
</tr>
<tr>
<td>2.** (4 expts.)</td>
<td>0</td>
<td>0</td>
<td>12.9±1.00</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td></td>
<td>7.0±0.90</td>
</tr>
<tr>
<td>1.0</td>
<td>0.1</td>
<td></td>
<td>11.7±1.66</td>
</tr>
</tbody>
</table>

* Insulin was added to the medium 15 minutes before phlorizin, and ** 30 minutes before phlorizin.

4) Effect of ouabain on the anaphylactic histamine release from guinea-pig lung tissue

Ouabain in concentration of 0.01 mM produced a marked reduction in the histamine release induced by antigen under nitrogen, in the presence of glucose in the medium. But this inhibitory effect of ouabain was also observed, though slightly less marked, in the histamine release under oxygen, irrespective of the presence or absence of glucose.
Oxygen uptake was slightly decreased only when glucose was present (Table 4).

Fig. 4 depicts the effects of graded concentrations of ouabain on the glucose-dependent anoxic histamine release, showing the effect still observable at the very low concentrations such as 10^-7 M.

**DISCUSSION**

As previously reported (3), the addition of succinate markedly enhanced the release of histamine by antigen from minced lung tissue of sensitized guinea pig. Glucose likewise showed a similar enhancing effect though only mildly. The anaphylactic histamine release from rat lung tissue, on the other hand, was not accelerated by succinate, but it was enhanced rather markedly by glucose. These findings agree well with those reported by Diamant (9). The problem that the effect of succinate differs in guinea pig and rat has to be left for further study since at present we have yet no satisfactory explanation for the exact mechanism of such an effect of succinate in guinea pig. Nevertheless, the enhancing effect of glucose on histamine release observed in the both species indicates that under oxygen exogenous glucose also plays a role of supplying the energy required for anaphylactic histamine release. Succinate induced an increase of oxygen uptake even with rat lung tissue in which it did not enhance the histamine release, a confirmation of the result by Diamant and Fredholm (10). In contrast, the increase of histamine release due to glucose was
not accompanied by any appreciable increase of oxygen uptake in either species. In other words, even if anaphylactic histamine release is taken as an oxygen requiring process, it is not necessarily a phenomenon which is quantitatively parallel to oxygen uptake. This argument, however, must take into consideration that the respiration being discussed here includes the respiration of tissue, in an enormously larger part, other than the cells (mast cells) that are directly concerned with histamine release.

The presence of glucose in the medium reversed almost fully the inhibition of histamine release by oxygen lack. The energy utilized for this anoxic histamine release must be provided by the products of glycolytic pathway. The increase of histamine release under oxygen as observed on addition of glucose may also practically be dependent on glycolytic process, because this increase was not accompanied by an increase in respiration.

The finding that the anaphylactic histamine release from guinea-pig lung tissue under anoxia induced by the presence of glucose was inhibited by 2-deoxyglucose coincides well with the report by Chakravarty (8). This inhibitor is phosphorylated by hexokinase, but it is thought that the phosphorylated product, 2-deoxyglucose-6-phosphate, accumulates inside the cell, and causes a block in the utilization of glucose by interfering with formation of glucose-6-phosphate (12, 13). This will explain why the histamine release to be induced by utilization of glucose was inhibited. It is, however, not so easy to explain the reason why the anaphylactic histamine release under oxygen was partially inhibited not only in the presence of glucose but also in the absence of it. It is not unreasonable to assume that where external substrate cannot be utilized, energy is supplied by break-down of intracellular glycogen and this process is blocked by accumulation of its product, 2-deoxyglucose-6-phosphate. However, the part of glycogenolytic energy supply, if any, would be too minimal to explain the inhibition of histamine release by this inhibitor, since in the medium free of external glucose histamine release was completely blocked by oxygen lack. Recently, Saeki (14) of this laboratory, who worked on degranulating action of Compound 48/80, α-chymotrypsin and anti-serum on the mast cells isolated from rat peritoneal fluid, found that the degranulation of mast cells by these histamine releasers was completely or largely inhibited by anoxia and uncoupler of oxidative phosphorylation just as in the case of anaphylactic histamine release, and glucose markedly blocked these inhibitions. In this experiment 2-deoxyglucose as well as phlorizin inhibited the anoxic degranulation of isolated mast cells elicited in the presence of glucose, but they, at the same concentrations in the present experiment, did not have any effect on aerobic degranulation. These discrepancies between the effects on mast cell degranulation and anaphylactic histamine release of 2-deoxyglucose may be due to the difference of the agents inducing reactions, or to some environmental variance of relevant mast cells, isolated or in situ.

Phlorizin is thought to interfere with the membrane permeability to glucose in a number of cell types by binding a glucose carrier with a high affinity at a membrane site of the cells (15). In the present experiment phlorizin inhibited glucose-dependent
histamine release rather markedly. The lower is the glucose concentration in medium, the greater is this inhibitory effect as was observed by Diamant (16). This can still more readily be understood from the premise that phlorizin competes with glucose for a monosaccharide carrier at the cell membrane, although opinions are at variance regarding whether phlorizin competes with glucose in a competitive or non-competitive way. The action of phlorizin differs from that of 2-deoxyglucose in that it did not show any significant influence on the aerobic histamine release.

Anaphylactic histamine release caused in the presence of glucose under anoxia was enhanced by insulin. This effect of insulin was marked when the glucose concentration was relatively high; this is favorable in interpreting this effect to be the result of increasing the quantity of glucose permeating the cell membrane. Manome and Kuriaki (17) reported that insulin promotes the absorption of glucose from the intestine of the rat and removes the inhibitory action on it of phlorizin. In the present experiment also insulin, in the concentration at which it does not accelerate anaphylactic histamine release by itself, almost completely blocked the inhibition of histamine release by phlorizin. This indicates that the effect of phlorizin and insulin on the anaphylactic reaction requiring glucose results from their effects on the membrane permeability of glucose.

Ouabain was also found to inhibit the glucose-dependent anaphylactic histamine release. There are some evidences indicating that ouabain inhibits the active transport of sugars across the intestine possibly linking with active ion transport (18, 19). This suggests a possibility that this inhibition of histamine release may also be the result of inhibition of glucose transport through the membrane of mast cells linking with ion transport mechanism in analogous manner. However, such an assumption may not be valid, since the inhibitory action of ouabain on histamine release was observed even in the glucose-free medium under oxygen. It seems to be dependent on a mechanism different from the inhibition of glucose transport. Notwithstanding, the glucose-dependent histamine release was inhibited in the same order of concentration ($10^{-7}$ M) as in the inhibition on the absorption of sugar (18) and on the fluxes of potassium and sodium ions across the cell membrane of human erythrocytes (20). Therefore, there is a possibility that the inhibition of active transport of ions, probably linking with any other process, still may affect the occurrence of anaphylactic histamine release. In this connection, it seems interesting to consider the possibility that anaphylactic histamine release, which involves energy metabolic processes and inhibited by several metabolic inhibitors known to more or less completely abolish the excitability of cells (21), may be interpreted as a consequence of cellular "excitation" characterized by specified ion shift, although yet decisive evidences are not available.

**SUMMARY**

1. Under oxygen in a substrate-free medium, anaphylactic histamine release from guinea-pig lung tissue was markedly enhanced by the addition of succinate but the
histamine release from rat lung tissue was not increased. Succinate evidently increased oxygen uptake irrespective of whether enhancing the histamine release or not. By glucose an increase in anaphylactic histamine release was produced in both species, but this was not accompanied by any significant increase in oxygen uptake in either species.

2. Glucose reversed the anoxic inhibition of anaphylactic histamine release from guinea-pig lung tissue. This glucose-dependent histamine release was inhibited by 2-deoxyglucose as well as phlorizin, and accelerated by insulin. Insulin removed the inhibition by phlorizin of the histamine release. Both 2-deoxyglucose and phlorizin showed no effect on respiration, but the former partially inhibited aerobic histamine release.

3. Ouabain inhibited the glucose-dependent anoxic histamine release in the same order of concentrations at which it inhibits active ion transport. But, this inhibitory action is not solely directed to the effect on glucose transport mechanism, because it also inhibited aerobic histamine release occurring in the absence of glucose.

Acknowledgement: Financial support for this investigation from the Ministry of Education, Japan for the Fundamental Scientific Research is gratefully acknowledged.

REFERENCES
1) MONGAR, J.L. AND SCHILD, H.O. : J. Physiol. 135, 301 (1957)
2) MOUSSATCHÉ, H. AND PROUVOST-DANON, A. : Experientia 14, 414 (1958)
3) YAMASAKI, H., MURAOKA, S. AND ENDO, K. : This Journal 10, 21 (1950)
4) CHAKRAVARTY, N. : Acta physiol. scand. 48, 146 (1960)
5) DIAMANT, B. : Ibid. 55, 11 (1962)
9) DIAMANT, B. : Acta physiol. scand. 56, 103 (1962)
10) DIAMANT, B. AND FREDHOLM, B. : Ibid. 59, 193 (1963)
14) SAEKI, K. : This Journal 14, 375 (1964)
16) DIAMANT, B. : Acta physiol. scand. 56, 97 (1962)