ABSENCE OF BLOCKING EFFECT OF BURIMAMIDE AND METIAMIDE ON POSITIVE CHRONOTROPIC AND INOTROPIC RESPONSES TO HISTAMINE IN ISOLATED DOG ATRIUM

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Abstract—When histamine was injected into the sinus node artery of isolated dog atrium perfused with arterial blood led from a carotid artery of the heparinized support dog, positive chronotropic and inotropic effects were dose-relatedly induced at a dose range of 0.1 to 100 μg. These positive responses to histamine were not blocked by tetrodotoxin, desmethylimipramine and alprenolol. These positive effects of histamine were also not significantly influenced by treatment with a histamine H2 receptor antagonist, burimamide or metiamide. However, these histamine-induced positive chronotropic and inotropic effects were significantly suppressed by an adequate dose of histamine H1 receptor antagonist, tripelennamine or diphenhydramine, which enhanced the actions of norepinephrine. From these results, it is suggested that in the dog atrium, histamine causes positive chronotropic and inotropic effects via histamine H1 receptors.

Black et al. (1) have recently classified histamine receptors into histamine H1 and H2 receptors. Histamine H1 receptors mediate most effects of histamine and are blocked by classical antihistamine compounds. On the other hand, histamine H2 receptors appear to mediate the gastric secretory and cardiac stimulatory effects of histamine and are not blocked by H1 receptor antagonists. In recent studies, burimamide and metiamide have been defined as histamine H2 receptor antagonists since they were found capable of blocking some mepyramine-insensitive histamine responses, such as the positive chronotropic effect on guinea pig atria, and the positive inotropic effect of guinea pig ventricles (1-4). However, in isolated dog heart, there is no available report for existence of histamine H2 receptors. Thus, in the present experiments, an attempt was made to investigate the characteristics of cardiac histamine receptors in isolated dog atrium by means of the selective histamine H2 receptor blocking agents, burimamide or metiamide, using the isolated, blood-perfused atrium preparation of the dog (5, 6). Moreover, the action of histamine was analysed by use of pharmacological key drugs, i.e., histamine H1 receptor antagonists (tripelennamine and diphenhydramine), alprenolol which is a potent adrenergic beta-receptor blocking agent, desmethylimipramine which blocks uptake mechanism in the sympathetic nerve terminals, and tetrodotoxin which blocks nerve excitation.

MATERIALS AND METHODS

Twenty-five mongrel dogs, weighing 11–16 kg, were anesthetized with sodium pen-
tobarbital, 30 mg/kg i.v. The isolated right atrium was perfused through the cannulated sinus node artery with arterial blood led from a heparinized support dog by aid of a peristaltic pump (Harvard Apparatus). Perfusion pressure was kept constant at 100 mm Hg. The atrium was suspended in the bath filled with blood at a constant temperature of 37°C. Isometric tension development was measured with a force displacement transducer (Grass FT03B). The maximum rate of tension development ($dT/dt$) was also recorded. Sinus rate was measured with a tachometer triggered by the wave of atrial electrograms. Details of the preparation have been described in previous papers (5, 6).

The flow rate at 100 mm Hg was 2–6 ml/min in all preparations. The muscle was usually subjected to a tension of 2 g. Drugs used in this study were histamine dihydrochloride (Wako), burimamide (Smith, Kline & French), metiamide (Smith, Kline & French), tripelemamine hydrochloride, diphenhydramine hydrochloride (Kowa), (+)-norepinephrine hydrochloride (Sankyo), nicotine (base), tetrodotoxin (Sankyo), alprenolol hydrochloride (AB Hässel), desmethylimipramine hydrochloride (Fujisawa) and tyramine hydrochloride (Wako). The volume of injected drug solutions was 0.01–0.03 ml in a period of 4 sec using a microinjector (Terumo Co.).

RESULTS

Effect of histamine on the isolated dog atrium

When histamine was injected into the cannulated sinus node artery, a positive chronotropic and inotropic effect was dose-relatedly induced as shown in Fig. 1. The threshold dose for inducing positive chronotropic and inotropic effect was approx. 0.3–1 μg. Relatively larger doses of histamine frequently induced two peaked positive chronotropic and inotropic responses, i.e., initially rapid responses followed by secondary long-lasting ones. In several cases, a positive inotropic effect was not so clear even at larger doses of 100 μg. Summarized data are shown in Table 1.

Absence of blocking effect of tetrodotoxin or desmethylimipramine on actions of histamine

When nicotine was given into the sinus node artery, negative chronotropic and inotropic effects followed by positive effects were dose-relatedly induced. These nicotine actions were inhibited by treatment with tetrodotoxin, which blocks neural excitation on the canine atrium as previously reported (7, 8). Histamine-induced positive chronotropic and inotropic effects were not influenced by treatment with tetrodotoxin. Summarized data are shown in Table 2 (A).

When desmethylimipramine was injected into the sinus node artery, negative chronotropic and inotropic effects followed by positive effects were dose-relatedly induced (9).
TABLE 1. Effects of increasing doses of histamine on SA nodal pacemaker activity and atrial contractility in the isolated atrium preparation

| Dose of histamine (µg) | Number of exp. | Maximum increase in sinus rate (%) | Maximum increase in contractile force (%) |
|-----------------------|----------------|------------------------------------|------------------------------------------|
| 0.3                   | 6              | 3.5 ± 1.2                          | 9 ± 3.0                                  |
| 1                     | 6              | 7.5 ± 1.8                          | 26 ± 5.9                                 |
| 3                     | 5              | 11.8 ± 2.5                         | 43 ± 8.6                                 |
| 10                    | 6              | 14.2 ± 1.4                         | 50 ± 9.8                                 |
| 30                    | 6              | 18.0 ± 3.5                         | 54 ± 10.1                                |
| 100                   | 4a             | 27.3 ± 7.6                         | 92 ± 13.3                                |

Values are mean ± SEM. The control sinus rate was 106 ± 4.9 beats/min (mean ± SEM) in 6 preparations. *In one out of four preparations, 100 µg of histamine caused sinus arrest. Thus, these values were obtained in 3 preparations.

TABLE 2. Effects of tetrodotoxin (A), desmethylimipramine (B) and alprenolol (C) on positive chronotropic (PCE) and positive inotropic (PIE) effects of histamine, nicotine, tyramine and norepinephrine

| Compound & dose | Before treatment | After treatment |
|-----------------|------------------|-----------------|
| (A) Tetrodotoxin treatment (3–10 µg, N=3) | | |
| Histamine 10–30 | 14 ± 4.0         | 12 ± 6.1*       | 40 ± 12.4e |
| Nicotine 3–10   | 20 ± 3.2         | 5 ± 1.1**       | 8 ± 3.0*** |

(B) Desmethylimipramine treatment (30–100 µg, N=4)

| Histamine 10–30 | 11 ± 3.1         | 6 ± 3.3*        | 52 ± 20.8* |
| Tyramine 0.3–3  | 17 ± 4.3         | 5 ± 1.2**       | 10 ± 2.1** |

(C) Alprenolol treatment (3–10 µg, N=5)

| Histamine 10–30 | 15 ± 2.1         | 18 ± 3.3*       | 36 ± 8.8* |
| Norepinephrine 0.01 | 35 ± 7.7        | 5 ± 2.7****     | 17 ± 9.3**** |

Values are mean ± SEM. Each control sinus rate was 111 ± 6.0 beats/min in 3 preparations (A), 110 ± 9.4 beats/min in 4 preparations (B) or 101 ± 7.1 beats/min in 5 preparations. Comparisons with control values (t-test): *P > 0.05, **P < 0.05, ***P < 0.01, ****P < 0.005.

When tyramine are administered into the sinus node artery, positive chronotropic and inotropic effects were observed. These tyramine-induced effects were inhibited by treatment with desmethylimipramine. However, after desmethylimipramine treatment, histamine-induced actions were slightly suppressed, although not significantly. Summarized data are shown in Table 2 (B).
Effect of alprenolol on actions of histamine and norepinephrine

When alprenolol, one of potent adrenergic beta-blocking agents, was injected into the sinus node artery, negative chronotropic and inotropic effects were dose-relatedly induced. Maximum percent increases in sinus rate and in the tension developed with histamine were not significantly suppressed by treatment with alprenolol which blocked the action of norepinephrine as shown in Fig. 2. Summarized data are shown in Table 2 (C).

Absence of blocking effect of burimamide or metiamide on histamine-induced actions

When burimamide was injected into the sinus node artery, positive chronotropic and inotropic responses were usually induced. Histamine-induced positive chronotropic and inotropic effects were not significantly influenced by burimamide treatment. Summarized data are shown in Table 3 (A).

When metiamide was injected into the sinus node artery, initially negative chronotropic and inotropic effects followed by secondary positive effects were induced. A large dose level of metiamide, 1 mg, also did not influence responses to 10 µg of histamine. Fig. 3 shows an example of the absence of blocking effect of metiamide on histamine-induced responses. Summarized data are shown in Table 3 (B).

Positive chronotropic and inotropic responses to 1 mg of burimamide or 1 mg of metiamide were completely blocked by treatment either with 10 µg of alprenolol or with 30 µg of desmethylimipramine in 2 experiments each.

Effects of tripelennamine and diphenhydramine on actions of histamine and norepinephrine

When tripelennamine or diphenhydramine was injected into the sinus node artery,

| Compound & dose | Before treatment | After treatment |
|-----------------|-----------------|----------------|
| (µg)            | PCE (%)         | PIE (%)        | PCE (%) | PIE (%) |
| (A) Burimamide treatment (1 mg, N = 5) |  |  |  |  |
| Histamine 3-10  | 12.0±2.1        | 32±7.1         | 10.5±2.2 | 28±8.0* |
| (B) Metiamide treatment (1 mg, N = 5) |  |  |  |  |
| Histamine 10    | 8.8±1.7         | 30±7.7         | 9.4±2.8* | 34±6.6* |

Values are means±SEM. Each control sinus rate was 102±4.1 beats/min or 110±6.0 beats/min in each 5 isolated atrium preparations. Comparisons with control values (t-test): *P > 0.05.
Table 4. Effects of tripelennamine (A) and diphenhydramine (B) on positive chronotropic (PCE) and positive inotropic (PIE) effects of histamine and norepinephrine

| Compound & dose | Before treatment | After treatment |
|-----------------|-----------------|----------------|
|                 | PCE (%)        | PIE (%)        | PCE (%) | PIE (%) |
| (A)             |                |                |         |
| Histamine       |                |                |         |
| 10–30           | 17±2.6         | 34±6.3         | 5±1.9***| 13±5.1**|
| Norepinephrine  | 22±0.6         | 173±442.0      | 27±5.3* | 232±74.0*|
| (B)             |                |                |         |
| Histamine       | 12±2.2         | 38±5.8         | 2±0.4***| 16±5.4**|

Values are mean ± SEM. Each control sinus rate was 108±7.2 beats/min in 4 preparations (A) or 100±3.2 beats/min in 5 preparations (B). Comparisons with control values (t-test): *P<0.05, **P<0.02, ***P<0.01, ****P<0.001.

Fig. 3. Effects of diphenhydramine and metiamide on actions of norepinephrine (NE) and histamine (H) in an isolated atrium preparation of the dog.

DISCUSSION

Recently it was reported that histamine exerts its cardiac effect by stimulation of a histamine H2 receptor, as histamine-induced positive chronotropic and inotropic effects were blocked by either burimamide or metiamide, a selective histamine H2 receptor antagonist (1-4). These results were obtained in experiments on guinea pig heart. More recently, it was also reported that in guinea pig atria, histamine increases myocardial contractility by an interaction with receptors closely related to classical histamine H1 receptors while its chronotropic effect is mediated by interaction with histamine H2 receptors (10, 11). In 1960, Trendelenburg (12) reported that chronotropic and inotropic responses to histamine by isolated atria were not effectively blocked by most of the widely used antihistamines.
This author used guinea pig, rabbit and cat atria and described that surprisingly large amounts of two antihistamines (pyrilamine and tripelennamine) were required to reduce the response to histamine. However, in this paper even a large amount of tripelennamine did not suppress the response to histamine in guinea pig atria, although such was obviously suppressed in rabbit and cat atria. Such findings suggest species differences in histamine receptors. In 1968, Dean (13) reported that pyribenzamine was found to be a specific antagonist for histamine on rabbit atria. In 1972, Hughes and Coret (14) reported that promethazine blocked the chronotropic and inotropic effects of histamine in isolated rabbit atria. However, they also suggested in another paper (15) that promethazine's blocking effectiveness may encompass both histamine H₁ and H₂ actions of histamine rather than being restricted to one or the other. In 1966, Flacke et al. (16) reported that antihistamine agents (promethazine and diphenhydramine) attenuated but did not block the effects on heart rate and contractility in the dog heart-lung preparation. More recently, Powell and Brody (17) reported that in intact dogs, histamine caused a positive chronotropic effect via histamine H₁ receptors, although they could not rule out the reflex mechanism. On the other hand, in the isolated dog heart there is no available report concerning effects of histamine H₂ receptor antagonist, burimamide or metiamide, on histamine-induced cardiac stimulation. In the present study, it was clearly demonstrated that positive chronotropic and inotropic effects of histamine were not influenced by burimamide or metiamide. Moreover, histamine-induced positive effects were significantly suppressed by a H₁ receptor antagonist, tripelennamine or diphenhydramine in this isolated atrium. Thus, it is suggested that histamine causes positive chronotropic and inotropic effects via histamine H₁ receptors in the dog atrium.

In inotropism, it is known that sinus acceleration causes an increase in the developed tension. The frequency-tension relationship of the dog atrium has already been reported by the author (18). Within a frequency range of 100 to 200 beats/min, a 20% increase in sinus rate caused about a 7% increase in the tension development. In the present study, however, the percent increase in the developed tension by histamine was much larger than that the sinus rate. Therefore, the histamine-induced positive inotropic effect may not be due to the positive chronotropic effect.

In 1954, Went et al. (19) reported that histamine might release catecholamines in guinea pig hearts. Flacke et al. (16) showed that in the dog heart-lung preparation, higher doses of histamine caused an increase in heart rate which was antagonized by propranolol. In the present study, given doses of histamine caused positive chronotropic and inotropic responses which were not significantly modified by the adrenergic beta-receptor blocking agent, alprenolol, the uptake blocking agent, desmethylimipramine, or the blocking agent of neural excitation, tetrodotoxin. Therefore, histamine apparently does not cause a release of catecholamine in the dog heart.

It was reported that burimamide causes positive chronotropic and inotropic effects by releasing catecholamines from adrenergic nerve terminals (20, 21). In the present study, it was demonstrated that both burimamide and metiamide exert cardiac stimulating proper-
ties via the tyramine-like action, as burimamide usually caused positive chronotropic and inotropic effects which were completely blocked by alprenolol or desmethylimipramine (7, 22). Moreover, although metiamide induced positive chronotropic and inotropic effects less potent than burimamide, these metiamide-induced positive effects were also inhibited by alprenolol or desmethylimipramine, indicating its tyramine-like action.

After tripelennamine treatment, histamine-induced effects were significantly suppressed but norepinephrine-induced effects were obviously enhanced. This enhancement may be due to a cocaine-like effect of tripelennamine as previously reported by Isaac and Goth (23).

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