POTENTIATING EFFECTS OF OUABAIN AND AMINOGUANIDINES ON RESPONSES OF SMOOTH MUSCLE ORGANS INDUCED BY VARIOUS AGENTS AND ELECTRICAL STIMULUS

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There are reports that cardiac glycosides potentiate responses of smooth muscle organs evoked by various stimulating agents as well as electrical stimulus. Brender and co-workers (1) reported that tension development and the contractile response of saphenous vein strip of dogs evoked by electrical stimulation were potentiated by acetylstrophanthidin (1 μg/ml). There is also evidence that digitoxin and ouabain potentiated the pressor actions of catecholamines in the dog (2,3) and that ouabain potentiated the response of aortic strip of the rabbit to BaCl₂ (4).

On the other hand, the authors synthesized several aminoguanidine analogues and examined pharmacological actions on autonomic nervous systems (5). Among them 3,4-dihydroxy-5-methoxybenzoylamino-guanidine (AG-2H) had characteristic cardiac action as follows (6). Namely, AG-2H showed a fairly continuous positive inotropic action without a positive chronotropic action. This action was difficult to abolish using an adrenergic β-blocking agent. The positive inotropic action induced by noradrenaline was abolished by a high concentration of AG-2H. As to action potential of guinea-pig papillary muscle, the plateau phase was prolonged by AG-2H though the amplitude was little changed.

In the present study, effects of ouabain and AG-2H on the responses induced by various agents and electrical nerve stimulation on the blood pressure and the nictitating membrane of the cat were examined in parallel. Furthermore, using guinea-pig vas deferens as a typical adrenergically innervated organ, effects of both drugs on contractions induced by autonomic agents were investigated.

METHODS AND MATERIALS

1) Blood pressure and nictitating membrane of the spinal cat

The spinal cat (2.0–4.5 kg) was prepared according to the method of Kumagai et al. (7).
Blood pressure was recorded through mercury manometer from femoral artery. Contractions of the right nictitating membrane were recorded on a smoked paper with an isotonic writing lever. Drugs dissolved in physiological saline were injected into the femoral vein through a cannula in a volume of approx. 0.3 ml. Ouabain and AG-2H were injected repeatedly during 30 min. Total doses were 20 μg/kg and 20 mg/kg, respectively.

2) Nictitating membrane of the anaesthetized cat

Nictitating membrane of cats (2.0-4.5 kg) which had been anaesthetized with urethane (1.4 g/kg s.c.) was used. Contractions of nictitating membrane evoked by tyramine and electrical stimulation of the post ganglionic fiber of the superior cervical sympathetic nerve trunks were recorded on smoked paper with an isotonic writing lever. Electrical stimulation using a Nihon Koden MSE-3 stimulator was applied at 3 min intervals with a frequency of 20 Hz, 1 msec duration and at submaximal voltage. In order to avoid influence of drugs on superior cervical ganglion, injections were made into the external carotid artery through the cannula, retrogradely, from the lingual artery, usually in a volume of 0.2 ml. Ouabain and AG-2H were injected repeatedly during 30 min. Total doses were 2 μg/animal and 2 mg/animal, respectively. Test compounds had been dissolved in physiological saline.

3) Guinea-pig vas deferens

After the vas deferens had been dissected, the preparation was suspended in a 10 ml organ bath containing a Tyrode solution (sodium chloride 137 mM, potassium chloride 2.7 mM, calcium chloride 1.8 mM. magnesium chloride 1.1 mM, sodium bicarbonate 11.9 mM, sodium dihydrogenphosphate 0.4 mM and glucose 5.6 mM) at 32°C. Contractions evoked by autonomic drugs (NA, tyramine, isoproterenol and ACh) were recorded isotonically on smoked paper. Due to cumulative properties of ouabain and AG-2H, the dose response curve could not be obtained. In cases of tyramine, isoproterenol, ACh in the normal preparation and NA in the cold storage preparation, the experiments were performed with the ED_{50} of these drugs. Within 30 sec after addition of ouabain (10^{-11}-10^{-10} g/ml) or AG-2H (5 x 10^{-8} g/ml), an agonist was added, then the preparation was washed three times with Tyrode solution. After this addition and washing had been repeated several times, maximum potentiating effect for each a gonist evoked by ouabain or AG-2H was obtained as in Figs. 5, 6 and 7. Cold storage of the vas deferens preparation was obtained after maintenance in 500 ml of Tyrode solution at 6-8°C for 7 days (8). All drugs were dissolved in Tyrode solution.

The level of significance was calculated using Student’s t-test (p=0.05). In these experiments, the following drugs were used: dl-noradrenaline hydrochloride (NA), tyramine hydrochloride, 1-isoproterenol, acetylcholine chloride (ACh), g-strophantin (ouabain) and 3,4-dihydroxy-5-methoxybenzoylaminoguanidine hydrochloride (AG-2H).

RESULTS

I Blood pressure and nictitating membrane of the spinal cat

Pressor response and the contraction of the nictitating membrane evoked by NA (0.1, 0.3, 1.0, 3.0 and 10.0 μg/kg, i.v.) and tyramine (0.1 mg/kg, i.v.) were potentiated by prein-
jection of ouabain (20 μg/kg, i.v. as total dose) or AG-2H (20 mg/kg, i.v. as total dose), shown in Figs. 1 and 2, respectively. These supersensitivities appeared 30-60 min after addition of ouabain and AG-2H, and lasted for several hr. Pressor activity of NA (10

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**Fig. 1.** Effects of ouabain on pressor responses and contractions of nictitating membrane evoked by NA and tyramine in spinal cat.

NA (0.1, 0.3, 1, 3 and 10 μg/kg) were intravenously administered at closed circles in order from left.

Tyramine (0.1 mg/kg, i.v.) was administered at open circles.

Ouabain (20 μg/kg, i.v. as total dose) was administered at arrow point.

Interval between (a) and (b) was 60 min.

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**Fig. 2.** Effects of AG-2H on pressor responses and contractions of nictitating membrane evoked by NA and tyramine in spinal cat.

NA (0.1, 0.3, 1, 3 and 10 μg/kg) were intravenously administered at open circles in order from left.

Tyramine (0.1 mg/kg, i.v.) was administered at closed circles.

AG-2H (20 mg/kg, i.v. as total dose) was administered at arrow point.
Fig. 3. Influence of AG 2H to pressor response of NA in spinal cat.
Abscissa: dose (µg kg, i.v.) of NA.
Ordinate: pressor response of NA expressed following value,
value mmHg in each dose of NA mmHg in 10 µg kg of NA without AG 2H.
Each point represents the mean value for 4 animals. Upper dotted line was
prepared with maximum potentiated response of NA obtained 30-60 min after
application of AG 2H (20 mg kg, i.v. as total dose). Vertical bars represent
standard errors.

Fig. 4. Effects of ouabain and AG 2H on contractions of nictitating membrane
evoked by electrical stimulation and tyramine in urethanized cat.
Electrical stimulation was applied on postganglionic fiber of the cervical
sympathetic nerve (frequency 20 Hz, duration 1 msec, submaximal voltage) at
closed circles.
Tyramine (0.1 mg animal, i.a.) was administered at open circles.
Ouabain (2 µg animal, i.a. as total dose) was administered at black arrow
point.
AG 2H (2 mg animal, i.a. as total dose) was administered at white arrow
point.
(a) and (c): control, (b) and (d): 30 min after administration of ouabain
and AG 2H.
tig/kg) was expanded 1.3-fold by the preinjection of ouabain and 1.5-fold by AG-2H. Dose response curve in the latter case is shown in Fig. 3. Difference in values between responses induced by each dose of NA before and after addition of AG-2H was statistically significant (p<0.05).

2 Nictitating membrane of the anesthetized cat

Contractions of the nictitating membrane of the urethane anaesthetized cat evoked by tyramine (0.1 mg/animal, i.a.) and the post ganglionic electrical stimulation (20 Hz, 1 msec, submaximal voltage) were potentiated by the intraarterial local application of ouabain (2 μg/animal) and AG-2H (2 mg/animal). This potentiation usually appeared 15 min after administration of ouabain and AG-2H, and response increased gradually reaching a maximum after 30-60 min (Fig. 4).

3 Guinea-pig vas deferens

NA: Effects of low concentration of ouabain and AG-2H on contractile response to NA (10⁻¹, 3x10⁻², 10⁻¹, 3x10⁻² and 10⁻⁵ g/ml) of the guinea-pig vas deferens, were examined. Responses to three concentrations (10⁻¹, 3x10⁻² and 10⁻⁵ g/ml) of NA were significantly potentiated by repeated application of ouabain (10⁻⁵ g/ml) and AG-2H (5x10⁻⁶ g/ml) (p<0.05). However, responses to the last two concentrations (3x10⁻² and 10⁻¹ g/ml) of NA were little potentiated by either compound. In the case of the lowest concentration

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**Fig. 5. Influence of ouabain on contraction of guinea-pig vas deferens evoked by NA.**

(a) Abscissa: concentration (g/ml) of NA.

Ordinate: response of vas deferens in the presence of a potentiator expressed as ratio (%) for control response (100%).

Vertical bars represent standard errors.

(b) An example of response to NA (3 x 10⁻⁵ g/ml) in the presence of ouabain (10⁻⁵ g/ml).

Dotted columns of (a) were prepared with 6 preparations using a maximum potentiated response shown in (b).

N: Number of experiments.
Fig. 6. Influence of AG 2H on contraction of guinea-pig vas deferens evoked by NA.  
(b) : An example of response to NA ($3 \times 10^{-5}$ g/ml) in the presence of AG-2H ($5 \times 10^{-5}$ g/ml).  
See Fig. 5 for details.

Fig. 7. Influences of ouabain and AG 2H on contraction of guinea-pig vas deferens evoked by tyramine.  
(a) Left : tyramine ($10^{-5}$ g/ml) control.  
Middle : AG 2H ($5 \times 10^{-5}$ g/ml) + tyramine ($10^{-5}$ g/ml).  
Right : ouabain ($10^{-6}$ g/ml) + tyramine ($10^{-5}$ g/ml).  
(b) : An example of response to tyramine ($10^{-5}$ g/ml) in the presence of ouabain.  
See Fig. 5 for details.

of NA, the potentiating effect of AG-2H was stronger than that of ouabain (Figs. 5 and 6).  
Experiments on every concentrations of NA were respectively performed with the six preparations.

Tyramine: Contractile responses of the vas deferens evoked by tyramine ($10^{-5}$ g/ml)  
were markedly potentitated by repeated application of ouabain ($10^{-6}$ g/ml) and AG-2H  
($5 \times 10^{-5}$ g/ml). Effects of both drugs were statistically significant ($p < 0.05$). Potentiation
induced by AG-2H was somewhat greater than that induced by ouabain (Fig. 7).

*Isoproterenol*: Responses of the vas deferens evoked by isoproterenol were significantly potentiated by repeated application of ouabain (10^{-11} g/ml) and AG-2H (5 \times 10^{-6} g/ml) (p<0.05) (Fig. 8).

*ACh*: Responses induced by ACh (10^{-7} g/ml) were significantly potentiated by repeated application of ouabain (10^{-11} g/ml) and AG-2H (5 \times 10^{-6} g/ml) (p<0.05) (Fig. 9).

*Cold storage (NaCl)*: On cold storage preparation of the vas deferens, contractile respon-
TABLE 1. Influences of ouabain and AG-2H on responses of guinea-pig vas deferens induced by various agonists.

| Agonists            | Potentiators (g/ml) | AG-2H (5 x 10^{-6}) | Ouabain (10^{-11}-10^{-10}) |
|---------------------|---------------------|----------------------|----------------------------|
| Noradrenaline       | 10^{-5}             | 180.62±29.21         | 194.40±58.31               |
| Noradrenaline (cold storage) | 10^{-6}             | 150.37±10.53         | 155.52±7.75                |
| Tyramine            | 10^{-5}             | 354.35±66.93         | 197.13±30.23               |
| Isoproterenol       | 10^{-5}             | 144.36±10.05         | 139.21±11.32               |
| Acetylcholine       | 10^{-7}             | 121.38±11.92         | 149.92±12.68               |

Figures expressed as % (±S.E.) of control response induced by each agonist.

induced by NA (10^{-5} g/ml) were significantly potentiated by repeated application of ouabain (10^{-10} g/ml) and AG-2H (5 x 10^{-6} g/ml) (p<0.05) (Fig. 10).

Effects of ouabain and AG-2H on responses of the vas deferens evoked by various agonists are summarized in Table 1.

DISCUSSION

Recently, several papers relating to the potentiating effect of the cardiac glycoside on the response of the smooth muscle organs induced by drugs have been reported by Tanabe and his co-workers (2). It was demonstrated that digitoxin potentiated, in dogs, the height and duration of pressor response evoked by NA and adrenaline. Evidence that potentiation to the pressor action of adrenaline by ouabain was increased by adrenergic β-blocking agent, but not by α-blocking agent, was observed (3). From these results it was suggested that this potentiating effect could be caused by influences of ouabain to α-receptor. In the present in vivo study, the authors found that, in spinal cats, responses induced by NA and tyramine on blood pressure and the nictitating membrane were potentiated by ouabain. In order to avoid influence on the superior cervical ganglion, all test drugs were administered into the external carotid artery after a cannula had been inserted into the lingual artery toward the carotid artery. According to Hoffman and Ten Eick (9), ouabain (<60 µg/kg, i.v.) failed to increase the excitability of the cervical sympathetic nerve trunks. On the other hand, it is a well established fact that cocaine potentiates response to NA but not to tyramine regarding nictitating membrane and the blood pressure, as a result of inhibition of amine uptake into the nerve endings (10, 11). Several investigators reported that uptake of NA and metaraminol into the brain and heart slice were inhibited by ouabain (10^{-2}-10^{-4} M) (12), yet in our experiments, ouabain potentiated not only response to NA, but also to tyramine. It is presumed that this phenomenon is induced by a cause different from that of cocaine.

In further experiments, isolated vas deferens preparation, a typical adrenergically innervated tissue, was employed. Contractile responses induced by NA, tyramine, isoproterenol and ACh in this preparation were nonspecifically potentiated by ouabain. Moreover, on cold storage preparation (7 days) which impaired nerve function (13—15),
the potentiating effect of the drug for the response induced by NA still remained. From these results, it is suggested that this potentiation was caused neither by blocking of amine uptake by ouabain to the nerve element, nor by the increment of the sensitivity of α-receptive site. The possibility that this potentiation results mainly in increment of the exitability of tissue membrane, is considered. It is presumed that ion permeability of the membrane was changed by the drugs. To support this argument, it has been reported that contraction of the aortic trip of the rabbit evoked by BaCl₂ was potentiated markedly by ouabain (4). In further studies, a low concentration of cardiac glycosides (10⁻¹²-10⁻⁹ M) stimulated Na⁺-K⁺ activated and Ca²⁺ activated ATPase of the heart muscle (16, 17). Also, the low concentration of ouabain (10⁻¹¹-10⁻⁸ M) stimulated Na⁺-K⁺ activated ATPase of the chicken kidney, and then, stimulated NA transport in the renal tubular membrane (19). There is, however, little data concerning with the effect of ouabain in ion-transport of the vas deferens. On the other hand, AG-2H induced nonspecific potentiations similar to that observed with ouabain. From this evidence, it is possible that the mechanism of potentiation by AG-2H is similar to that by ouabain. In preliminary studies, though AG-2H failed to induce the contraction of the isolated guinea-pig vas deferens, the compound showed direct sympathomimetic action on the blood pressure of the cat and the rat (6). Therefore, it could increase to some extent excitability of α-receptive site of the vas deferens, even if AG-2H alone does not cause contractile response.

Effects of high concentration ouabain on responses of the vas deferens induced by these agents is now being investigated in our laboratory.

SUMMARY

Nonspecific potentiating effects evoked by low doses of ouabain and 3, 4-dihydroxy-5-methoxybenzoylaminoguanidine (AG-2H) to the responses of various agents on the smooth muscle organs were studied. Responses of NA and tyramine on blood pressure and the nictitating membrane of the spinal cat were gradedly potentiated by repeated application of ouabain (20 μg/kg, i.v. as total dose) or AG-2H (20 mg/kg, i.v. as total dose). Responses induced by tyramine and the submaximal electrical stimulation of postganglionic cervical sympathetic nerve trunks on the nictitating membrane of urethanized cats were similarly potentiated by intraarterial local application of the both compounds. In guinea-pig vas deferens, the contractions caused by NA, tyramine, isoproterenol and ACh were potentiated by low doses of ouabain and AG-2H. On cold storage preparation, also contraction evoked by NA was potentiated.

From these results, it can be considered that these potentiating phenomena were caused by the changing of ion (Ca²⁺ and Na⁺) transport at the cell membrane of the smooth muscle rather than the supersensitivity of only α-receptive site and the inhibition of amine uptake into the nerve element by ouabain and AG-2H.

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