EFFECT OF TAURINE ON RESPONSES TO NORADRENALINE, ACETYLCHOLINE AND OUABAIN IN ISOLATED AURICLES FROM DIGITALIZED GUINEA PIGS

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Abstract—Responses of the isolated heart from the guinea pig treated chronically with digitoxin until the T wave had disappeared were studied. The development of the abnormal ECG pattern was abolished by a simultaneous injection of taurine and digitoxin. Taurine also inhibited the change by digitoxin of the responses to acetylcholine and ouabain but not to noradrenaline. The causal relationship between the inhibitory effect of taurine and prevention of the development of arrhythmias remains obscure.

Taurine is a sulfur-containing amino acid, found in many tissues including the heart. Although its biological importance as taurocholic acid has already been established, the function of taurine as related to the heart is not clear.

Read and Welty (1) found that pretreatment with taurine prevented adrenaline- or digoxin-induced ventricular premature contraction in the dog. We also noted a similar effect in the rat (2). Namely, when taurine was infused together with ouabain, the development of arrhythmias was prevented. The decrease in taurine concentrations was seen in the heart with ouabain-induced arrhythmias. Propranolol inhibited both effects. Thus, it seems likely that the actions of the autonomic nervous system are attributable, at least in part, to the antiarrhythmic action of taurine.

It has been reported that the inotropic effects of ouabain are mediated through catecholamines (3), although there are reports which negate this (4, 5). The chronotropic effect is also reportedly dependent upon catecholamines released by the drug (6). Furthermore, according to Gillis et al. (7), not only cardiac sympathetic nerve activity but also parasympathetic activity was elevated with ouabain which also antagonizes the negative inotropic action of acetylcholine (8) and sensitizes S-A node and A-V node to acetylcholine (9, 10).

We attempted herein to examine whether taurine, a non-cholinergic and non-adrenergic drug, given in antiarrhythmic doses, modifies the change by digitoxin in the cardiac responses to noradrenaline, acetylcholine and ouabain.

MATERIALS AND METHODS

Male guinea pigs with an initial body weight of 100 to 120 g were divided into four
groups. Saline (0.2 ml) was injected i.m., 14 times every two days to the controls (Group 1). Digitoxin was administered i.m. at a dose of 0.1 mg/kg, 7 times at intervals of 4 days (Group 2). Taurine was given i.p. at a dose of 100 mg/kg, 14 times every two days (Group 3). To animals in the fourth group, digitoxin and taurine were injected in the same manner as in Groups 2 and 3, respectively. ECG (Lead II) of the unanaesthetized animal was recorded by electrocardiograph.

Spontaneous beating of the isolated auricles mounted in oxygenated Tyrode's solution at 32°C was isotonically recorded on smoked paper via a tension balance. The frequency was recorded after additions of noradrenaline and acetylcholine. In some experiments, muscle strips, about 1 cm long, 0.5 cm wide and 0.5 mm thick, were removed from the left atria of newly sacrificed guinea pigs and were suspended in organ baths containing oxygen-saturated Tyrode's solution at 32°C. The strip was driven by electrical stimulation (frequency: 1 cps, duration: 5 msec, output: 8 v) and the contraction amplitude was measured 1.25 min after acetylcholine and noradrenaline and 15 min after ouabain.

Taurine was extracted from the heart according to the method of Hope (11), purified by column and thin-layer chromatographies and determined colorimetrically as described by Guidotti et al. (12).

Drugs used were digitoxin, ouabain, noradrenaline (E. Merk, Darmstadt), taurine (Taisho Pharmaceutical Co., Ltd., Tokyo) and acetylcholine (Daiichi Seiyaku, Ltd., Tokyo).

RESULTS

In a preliminary experiment, when digitoxin was injected i.m. at a dose of 0.1 mg/kg, 5 times at intervals of 4 days, a gain in body weight was not significantly reduced and ECG patterns were not altered in all of 35 instances. Moreover, isolated cardiac preparations from these animals did not show abnormalities in the chronotropic and the inotropic responses to acetylcholine and noradrenaline.

On the other hand, when the i.m. injection of digitoxin at the same dose and the same intervals was repeated 7 times instead of 5 times, the gain in weight was significantly reduced (Fig. 1) and the T wave disappeared in all 35 instances (Group 2). When the auricles, isolated from the guinea pig with the abnormal ECG pattern were suspended in the

FIG. 1. Effects of digitoxin, taurine and digitoxin plus taurine on a gain in body weight. Digitoxin was injected at intervals of 4 days and taurine was at intervals of two days. ○, control (n=20); ●, digitoxin (n=35); △, taurine (n=25); ▲, digitoxin plus taurine (n=25). Bars indicate S.E. *significant difference from saline-treated groups (P<0.05).
Tyrode's solution, a spontaneous beating was as rhythmic as that of the control preparation and the number of the beatings was not significantly changed (Table 1). Further experiments, therefore, were done with digitoxin given 7 times.

Taurine did not produce the abnormal pattern of ECG and reduced the gain in weight but prevented the development of arrhythmias by digitoxin (Group 3).

The negative chronotropic action of acetylcholine at doses ranging from $10^{-8}$ M to $10^{-6}$ M was weakened in the digitoxin-treated group, Group 2 (Fig. 2), in which the negative inotropic effect at doses of $10^{-9}$ M to $10^{-6}$ M was potentiated (Fig. 3). Taurine alone did not modify the response to acetylcholine (Group 3). However, when digitoxin was adminis-

| Groups | Treatments         | No. of Exp. | No. of beats/min |
|--------|--------------------|-------------|------------------|
| 1      | Saline             | 27          | 118± 7*          |
| 2      | Digitoxin          | 22          | 103± 12          |
| 3      | Taurine            | 17          | 121± 8           |
| 4      | Digitoxin+Taurine  | 23          | 118±10           |

*Means±S.E.
stered together with taurine, the alterations by digitoxin of cardiac responses to acetylcholine were significantly depressed (Group 4).

Digitoxin augmented the positive chronotropic (Fig. 4) and inotropic responses (Fig. 5) to noradrenaline at doses less than $10^{-7}$ M (Group 2), and taurine partially increased these responses to the amine (Group 3). The treatment with digitoxin in the presence of taurine significantly increased the cardiac effect of noradrenaline (Group 4) when compared with the controls.

The positive inotropic action of ouabain at doses of $10^{-8}$ M to $2 \times 10^{-8}$ M was potentiated in Group 2, digitoxin-treated groups (Fig. 6). When taurine was injected simultaneously with digitoxin, the effect of ouabain remained unchanged (Group 4). Taurine alone did not vary the response to ouabain (Group 3).

The cardiac uptake of taurine was seen after i.p. injections of taurine (Table 2). Digitoxin did not alter the concentration of taurine in the heart nor influence

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**Fig. 4.** The positive chronotropic action of noradrenaline on the isolated auricles. Ordinate, % increase in the contraction frequency, abscissa, molar concentration ($-\log$ scale). Legends as in Fig. 2. Bars indicate S.E. of the mean in 11-19 experiments.

**Fig. 5.** Positive inotropic actions of noradrenaline on the left atrial strips. Ordinate, % increase in the contraction amplitude, abscissa, molar concentration ($-\log$ scale). Legends as in Fig. 2. Bars represent S.E. in 9-20 experiments.

**Fig. 6.** Dose-response curves for ouabain on the electrically driven left atrial strips. Ordinate, % change in the contraction amplitude, abscissa, molar concentration ($-\log$ scale). Legends as in Fig. 2. Bars indicate S.E. in 8-11 experiments.
DISCUSSION

Downward displacement of the S-T segment and inversion of the T wave could be clinically observed as manifestations of the early state of digitalis-intoxication (13) and we also noted abnormalities on ECG, such as the T wave-disappearance when i.m. injections of digitoxin at a dose of 0.1 mg/kg were repeated 7 times at intervals of 4 days. Using guinea pigs, we attempted to determine whether or not the digitoxin-intoxication influences cardiac responses to drugs, and acetylcholine, noradrenaline and ouabain were chosen as a cholinergic, adrenergic and a non-cholinergic and non-adrenergic drugs, respectively.

Since the gain in weight was reduced with this treatment, it should be kept in mind that abnormal responses to drugs in the digitoxin-treated group could be the result, at least in part, of a reduction in weight-gain.

It has already been established that the negative chronotropic effect of acetylcholine was potentiated in the dog (9) and the rabbit (10) and that the negative inotropic effect was inhibited by digitalis in rabbits (8). In our work with guinea pigs, we noted that the negative chronotropic effect of acetylcholine was attenuated and the negative inotropic effect was strengthened in the intoxicated guinea pig heart. Discrepancy between our results and those of others may be due to the highly-specific manner of digitalization in each species, since we digitalized the guinea pig with chronic administrations, while the other workers used acutely-digitalized preparations.

We were of the opinion that ouabain caused an abnormal contraction when added to digitalized and isolated hearts. The contraction of electrically-driven atrial strips and the spontaneous contraction of the right atrium were both normal in the digitoxin treated group, and while the abnormal contraction of the formar was not seen even after ouabain-addition in this group, the inotropic action of ouabain was rather potentiated suggesting that the digitalization may not be so severe and that action sites for ouabain may be sensitized by digitoxin.

The antiarrhythmic effect of taurine has been observed by Read and Welty (1), Chazov et al. (14) and by the present authors (2). Furthermore, in the present work, it was noted that taurine itself did not change either the ECG pattern or cardiac responses to acetylcholine and ouabain, but did prevent the development of arrhythmias with digitoxin. With pre-

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### Table 2. Comparison of the cardiac level of taurine in 4 groups

| Groups | Treatments        | No. of Exps | Cardiac level of taurine (μg/g) |
|--------|-------------------|-------------|--------------------------------|
| 1      | Saline            | 8           | 989±125                        |
| 2      | Digitoxin         | 4           | 885±63                         |
| 3      | Taurine           | 4           | 1338±50*                       |
| 4      | Digitoxin+Taurine | 2           | 1106, 1080                     |

* Significant (P<0.05) compared with Group 1
Values are given as means±S.E.

The increase in the cardiac uptake of taurine after the injection of taurine.
vention by taurine of digitoxin-arrhythmias, the responses of isolated hearts to acetylcholine and ouabain were restored to the control.

Myocardial Na+, K+-ATPase may not be involved in this antagonism by taurine, since the drug did not influence the enzyme activity or inhibition by ouabain of the activity in vitro (2).

Cardiac responses to noradrenaline were augmented by digitalis treatment. As reported by Gillis et al. (7), cardiac sympathetic nerve activity may have been increased as a result of the chronic injections of digitoxin. In addition, taurine potentiated responses to low doses of noradrenaline (Group 2). Noradrenaline may increase the concentration of taurine available in action sites which in turn would increase the inotropic response, since taurine has a positive inotropic action (15) and the accumulation of taurine in the heart was observed in taurine-treated groups. If such a postulation is valid, indeed the chronotropic effect of the amine may be increased by taurine. We did not pursue further the fact that the increase in the response to noradrenaline and in the myocardial level of taurine with the simultaneous treatment of digitoxin and taurine (Group 4) did not significantly differ from that in each of the digitoxin-treated and the taurine-treated groups.

Finally, the fact that changes in responses to acetylcholine, noradrenaline and ouabain were seen in part only at relatively low doses in the digitoxin-treated animal suggests that the development of arrhythmias did not necessarily follow the changed responsiveness of the heart. Since the effects of digitoxin on the heart are complicated, mechanisms for the antiarrhythmic action of taurine remain unelucidated. We did not observe a positive intra-relationship between the antiarrhythmic effect of taurine and prevention by taurine of digitoxin-induced changes in cardiac responses to drugs. Further experiments are required to determine whether the changes in response to the drug are associated with the development of arrhythmias and how taurine acts when a greater degree of digitalization is performed.

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