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# Analysis of the Inotropic Action of Ouabain in Rat Ventricles: Two Apparent Ouabain Inotropic Responses (41763)

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*Abstract.* Two distinct inotropic effects of ouabain were observed in 68 of 82 right ventricular strips of rats with $ED_{50}$s of 0.5 and 20 $\mu M$ referred to as "low-dose" and "high-dose" effects, respectively. The other 14 strips showed monophasic dose–response curves, with an apparent $ED_{50}$ of 0.3 $\mu M$; the inotropic response to ouabain or isoproterenol developed by these atypical strips did not exceed 50% over control. In the strips showing the biphasic inotropic response curves, the proportion of the "low-dose" effect varied from 6 to 89% of the maximum response. After treatment with 160 $\mu M$ ouabain, followed by 60 min of washout, the maximum developed tension in response to ouabain was unchanged, but the "low-dose" effect was abolished or dramatically reduced. In those cases with a remaining "low-dose" response, the response was only 12% or less of the maximum and the $ED_{50}$ was shifted from 0.3 to about 3 $\mu M$ ouabain by the washout. The atypical strips, which showed only the "low-dose" effects during the first ouabain exposure, revealed after washout a consistent "high-dose" effect with an $ED_{50}$ of about 12 $\mu M$ ouabain. The data show that the two inotropic responses exist in all ventricular strips and that in some strips, after ouabain washout, a residue of the "low-dose" effect remains.

The rat has long been recognized in toxicological studies as a digitalis-resistant species (1, 2). Recently, Erdmann *et al.* (3) reported that the inotropic response of isolated rat ventricular strips was elicited by surprisingly low concentrations of ouabain. The $ED_{50}$ for this effect, 0.3 $\mu M$, in fact, was orders of magnitude less than the concentrations of ouabain required to inhibit rat heart kidney $Na^+,K^+$-ATPase (between 10 and 100 $\mu M$) (2, 4, 5). The separation of inhibition of $Na^+,K^+$-ATPase and the inotropic effects in the rat heart seemed contrary to the proposal that the inotropic effect of ouabain is due to $Na^+,K^+$-ATPase inhibition (1, 2). However, we (6, 7) have found a biphasic dose–response curve in isolated rat ventricular strips but not in atria, with ouabain producing a so-called "high-dose" effect ($ED_{50}$ of 20 $\mu M$ and a "low-dose" effect $ED_{50}$ of 0.5 $\mu M$). We could show by appropriate exposure to blocking agents (6) that $\alpha$- or $\beta$-adrenergic or $H_2$-histaminic transmitter release is not involved in the generation of the "low-dose" effect. Thus, the ouabain dose–response curve constructed from the rat ventricle data spans more than 3 orders of magnitude compared to the more typical steep one found in the rat atrium with an approximate $ED_{50}$ of 55 $\mu M$ (6).

The inotropic effect of ouabain and its relationship to $Na^+,K^+$-ATPase inhibition remains somewhat controversial for digitalis-sensitive species as well as for the rat, but the rat ventricle is the only tissue where a clear-cut biphasic dose–response curve has been reported. To complicate matters, we observed that the "low-dose" effect disappeared when the ouabain dose–response curve was repeated a second time (6). We have referred to this phenomenon as desensitization. However, it is not known whether the apparent disappearance or desensitization is complete, in that some of the ventricular strips exhibited only a monophasic "low-dose" ouabain effect, the same monophasic effect reported by Erdmann *et al.* (3). In this paper we present the results of an extensive series of experiments in which the "low-dose" and "high-dose" effects can be demonstrated in all ventricular strips. Some strips retained a small "low-dose" effect after prolonged ouabain washout.

**Materials and Methods.** We used 82 right ventricular strips from 43 rat hearts (average body weight 250 g) for the experiments at 35°C in Tyrode solution (m$M$): 137 NaCl, 5.0 KCl, 1.8 $CaCl_2$, 1.05 $MgSO_4$, 0.42 $KH_2PO_4$, 11.9 $NaHCO_3$, and 10 glucose, pH 7.0; or Krebs Henseleit solution (m$M$): 118 NaCl, 4.7 KCl,



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2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 $KH_2PO_4$, 25 $NaHCO_3$, 11.5 glucose, 0.5 NaEDTA, pH 7.4, saturated with 95% $O_2$ and 5% $CO_2$. Strips from the right ventricle are preferred over those of the left because its wall is much thinner and, consequently, poses much less resistance to oxygen diffusion. We have shown in previous experiments (6) that, although there is a difference between rat atria and rat ventricles in the response to ouabain, there is no difference between right and left ventricle. The heart tissues were studied under isometric conditions (8) with an initial load of 1 g and stimulated at 1 Hz, stimulus duration 2 msec, and voltage 20% above threshold (usually 3 to 5 V). The resting tension did not change significantly during the experiments. The preparations were equilibrated for 60 to 120 min. For more details see (8). All dose–response curves were obtained on a cumulative basis and the $ED_{50}$ was determined graphically. Ouabain was added to the bath cumulatively every 10 min beginning with 0.1 to 160 $\mu M$. The 10-min exposure time was selected to be consistent with the observations of Erdmann *et al.* (3) that in rat ventricles at 35°C and 1 Hz, the maximal effect of ouabain was reached at or before 10 min. After a washout period of 30 to 120 min, the dose–response study was repeated. The values presented are means ± SEM.

**Results.** The positive inotropic effect of ouabain is a relatively fast process in the rat ventricle with the steady-state effect being reached in 10 min at the lowest concentrations of the drug (3). Cumulative concentrations of ouabain were applied over a large range of concentrations to allow a separation of the dose–response curves into the putative phases. In most cases, clear biphasic dose–response curves were obtained with a "low-dose" and a "high-dose" effect which were graphically estimated. However, a large variation in the types of curves was observed (Fig. 1, left panel). We decided, therefore, to pool the data arbitrarily into three groups: group A (triangles), where the "low-dose" effect represented between 1/3 and 2/3 of the maximum developed tension; group B (circles), where the "low-dose" effect represented practically all of the maximum effect, and group C (squares), where it represented less than 1/3 of the maximum effect. Of the 82 strips studied, 33 fell into group A (most typical biphasic curves), 23 strips into group B ("low-dose" effect predominant), and 26 into group C ("high-dose

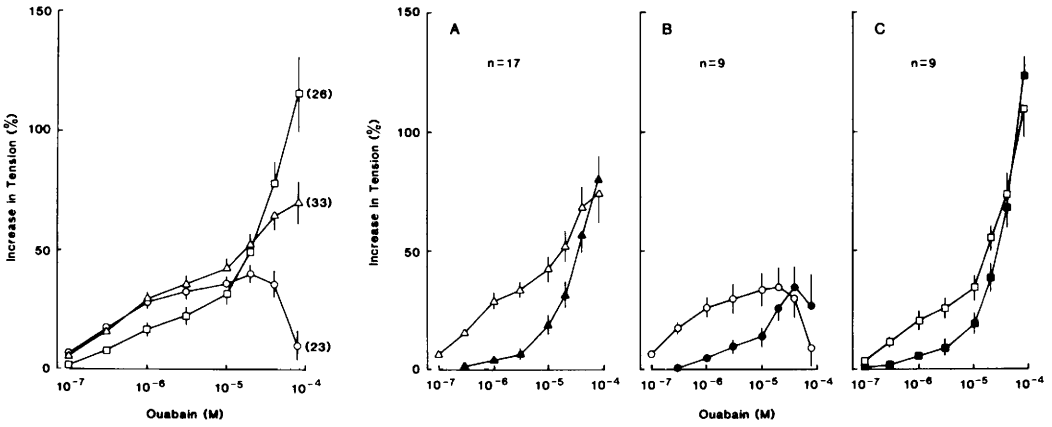


FIG. 1. Left panel. Inotropic responses of 82 rat ventricular strips to cumulative concentrations of ouabain expressed in percentage of the initial active tension. "Low-dose" effect was measured at $3 \times 10^{-6}$ $M$ ouabain, "high-dose" at maximum (maximal effect of ouabain). The dose–response curves were grouped according to their "low-dose"/"high-dose" relationship: group A (triangles), "low-dose" effect 1/3 to 2/3 of maximum; group B (circles) "low-dose" effect more than 2/3 of maximum; group C (squares) "low-dose" effect less than 1/3 of maximum. Panels A, B, and C. Intragroup comparison between two successive cumulative dose–response curves with ouabain separated by a 30- to 120-min washout period. After washout, the basal contractile force of all strips declined (mean 88.6 ± 0.4% of the original active tension, $N$ = 35). Open symbols: first response curve; closed symbols: second response curve. The points are means ± SEM.

effect predominant). Of the 23 dose–response curves in group B, 14 were found to be purely monophasic, i.e., exhibiting only the "low-dose" effect.

In order to identify sources of the variations, we compared the maxima of the "low-dose" and "high-dose" effects with the initial active tension of each strip measured before the addition of ouabain. We found that the maximum inotropic effects were proportional to the initial active tension of each strip. The mean initial active tension was 0.504 ± 0.057 g in group A, 0.396 ± 0.032 g in group B, and 0.401 ± 0.046 g in group C. Similarly, the "low-dose/high-dose" ratio varied in groups A and C between 0.07 and 2.1 in those strips able to develop a maximum inotropic response for more than 100% (mean 159 ± 19%, $N = 17$), and between 0.08 and 4.6 in strips with a maximum inotropic response of 50 to 100% (mean 69 ± 2%, $N = 31$). In group B, most of the strips developed a maximum response which was below 50%, while 13 out of 33 strips in group A and 2 out of 26 in group C also developed a maximum response which was below 50%. In 16 strips which comprise representative dose–response curves from all three groups, the maximum inotropic response to ouabain was compared with the maximum effect produced by isoproterenol. We found the two maxima about equal in rat ventricular strips (data not shown).

Toxicity developed at high concentrations of ouabain (80–160 $\mu M$) in the form of an increase in resting tension accompanied by a decrease in developed tension. However, in some strips a decrease in developed tension (without increase in resting tension) occurred at concentrations as low as 10 $\mu M$. In group A, 27% of the strips developed toxicity at 80 $\mu M$ and 73% at 160 $\mu M$ ouabain or higher. In group B, 29% of the strips developed toxicity at 10 and 20 $\mu M$, 54% at 40 and 80 $\mu M$, and 17% at 160 $\mu M$ ouabain or higher, whereas in group C, 4% of the strips showed toxicity at 80 $\mu M$ and 96% at 160 $\mu M$ ouabain or higher.

The desensitization phenomenon reported previously (6) was also observed in all three groups (Fig. 1, panels A, B, and C), but in 17 of the 35 strips it was incomplete. The maximum "low-dose" effect estimated at 3 $\mu M$ in these 17 strips was reduced from 36 ± 4 to 10 ± 2% and the $ED_{50}$ was increased from 0.3 to 3 $\mu M$. Of particular interest was the observation that the strips characterized by monophasic first response (type B, Fig. 1) showed a clear biphasic curve on the second exposure to ouabain. In these atypical strips which had developed early toxicity on first exposure (see paragraph above), toxicity appeared late on second exposure. Toxicity in the second dose–response curves did not occur below 160 $\mu M$ in groups A and C, and below 80 $\mu M$ in group B.

**Discussion.** It is apparent that the typical inotropic response of rat ventricular strips to ouabain is biphasic. This suggests the possible presence of two receptor forms with different affinities for ouabain (2, 7). However, it is also apparent that the ratio between the "low-dose" and the "high-dose" effects may vary considerably. For example, 14 of the 82 strips we used responded only to low concentrations of ouabain, revealing a monophasic pattern. This monophasic response, which was, in fact, infrequent in our study, closely resembled that reported originally by Erdmann *et al.* (3). These investigators, however, constructed their dose–response curves from single ouabain exposures per strip for 60 min. In order to simplify comparison of our results we grouped our results into three "types." Type C showed the clearest pattern of the two inotropic effects, with the "low-dose" $ED_{50}$ about 0.5 $\mu M$ and the "high-dose" about 25 $\mu M$ ouabain. In the Type A strips, the "low-dose" $ED_{50}$ was also about 0.5 $\mu M$, but the "high-dose" $ED_{50}$ varied from 5 to 25 $\mu M$. Toxicity in the Type A and C strips developed between 80 and 160 $\mu M$ ouabain. In Type B strips, the "low-dose" effects prevailed, with the $ED_{50}$ again about 0.5 $\mu M$ ouabain, with none or only an indistinct "high-dose" effect. However, when we repeated the dose–response experiments the "high-dose" effects became more clearly established even in the Type B strips (Type A 25 $\mu M$, B 12 $\mu M$, and C 39 $\mu M$ ouabain). On the other hand, during the second addition experiments, the "low-dose" effect was considerably decreased or abolished. This phenomenon was previously referred to as "desensitization" of a high-affinity receptor form (6). Experiments with $\alpha$ and $\beta$ blockers and $H_2$ antagonist showed (6) that these transmitters were not involved in the production

of the "low-dose" effect. The high-affinity form could also have been "converted" into a low-affinity form (9, 10). The increase in $ED_{50}$ observed for the "low-dose" effect, however, suggests the existence of multiple interconvertible forms. Desensitization was also observed in the atypical monophasic strips which showed only "low-dose" effects on the first exposure but a greatly reduced "low-dose" and a clear "high-dose" effect after washout. Therefore, two inotropic receptor forms can be demonstrated in all ventricular strips. It is of importance to emphasize that the atrial myocardium of the rat has only one phase in its dose–response curve of the "high-dose" type (6).

The maximum inotropic responses of the atypical ventricular strips which exhibited only "low-dose" effects to ouabain and, where determined, to isoproterenol, were relatively small and did not exceed 50% above control. Therefore, the physiological expression of the low-affinity "high-dose" receptor form may depend on the ability of the tissue to respond to exogenous inotropic agents with large increases. The appearance of a consistent "high-dose" effect after washout in atypical strips with weak inotropic potency suggests that the ratio between the two receptor forms in each strip could also be important. It should be noted that the atypical strips also revealed toxicity at rather low concentrations of ouabain (approximately 10 $\mu M$ instead of 80 to 160 $\mu M$). Therefore, the possibility has to be considered that these strips may have been mechanically or physiologically damaged during preparation.

There is the possibility that the disappearance of the "low-dose" effect, i.e., desensitization of ouabain in rat ventricular strips, is caused by low concentrations of ouabain ($<3$ $\mu M$) which remained attached to the high-affinity receptor site. The 35 washout experiments reported in this paper could be used as pharmacological evidence for or against the presence of ouabain in the strips, similar to the concept developed by Hatcher and Bailey (11) using a bioassay of cardiac glycosides. In all experiments, the basal contractile force was reestablished after a 30- to 120-min washout of the effects of increasing concentrations of ouabain (0.1 to 160 $\mu M$) on contractile force of the rat ventricular strips. In fact, there was a small decline of basal contraction of about 12% compared to the contractile force in the beginning of the experiments. The repetition of the dose–response curves revealed a normal response of the strips to the "high-dose" effect of ouabain with the maximum of inotropic effect and the minimum of toxic concentration similar to those recorded from the first dose–response curve. The interaction of ouabain with the receptors which mediate the "high-dose" effect and the toxic effect is undoubtedly a rapid dynamic process since the association and dissociation rates are both fast in membranes from the rat heart (5). The same rapid onset and washout of the positive inotropic effects are seen in isolated muscle preparations from the rat heart (6). This is not the case with the "low-dose" effect. The concentrations of ouabain involved in this effect are much smaller, about 0.1 to 3 $\mu M$, and only a few percent of the "high-dose" concentrations. However, pharmacologically the "low-dose" effect can no longer be elicited with ouabain after its receptor has been in contact with large concentrations of ouabain. If the residual drug would still be occupying the "low-dose" receptor, the "low-dose" positive inotropic effect would have continued after washout. Instead we observed after washout of ouabain a return to or below the control basal contractile force. Our pharmacological evidence supports, therefore, the notion of desensitization of the "low-dose" high-affinity receptor or a conversion of the high-affinity site to one of the lower affinity, or the existence of multiple interconvertible receptor forms.

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