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Timed dose-response relationship of depressive action of ouabain on toad heart contraction *in vitro*[1]

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ABSTRACT Timed dose-response relationship (TDRR) of depressive action of ouabain (Oua) 0.01 —30 mmol·L^{-1} on toad heart contraction *in vitro* was studied by simultaneous recordings of mechanical tension and electrocardiography. It was found that the inhibition of contraction tension, the latency, the half equilibrium time of depression, and the cardioplegia time (CPT) were all concentration-dependent ($P < 0.01$). The $EC_{50} \pm L_{95}$ of Oua was 35 ± 8 μmol·L^{-1}, in which L_{95} is the average 95% confidence limit. The TDRR data of latency or CPT were fitted to the hyperbolic 4-parameter model II weighted with the square of SE inversely; $\hat{Y} = 25.9492^{7.2757}/(\ln X + 23.5906)^{6.2757} + 72.7732$ for latency and $\hat{Y} = 20637.37/(\ln X + 3.0947)^{3.5907} + 196.7069$ for CPT. It was concluded that the hyperbolic type of TDRR was present in Oua depressive action on the toad heart contraction *in vitro*.

KEY WORDS ouabain; heart; drug dose-response relationship; electrocardiography; time; regression analysis

Although the actions of ouabain (Oua) on cardiac functions have been studied for years, the time appearance[1] of the actions has not been fully elucidated. Our previous study showed that some timed responses such as latency of Oua action were concentration-dependent[2]. But, what kind of TDRR[1,3-7] is it? What type of quantitative relation exists between the timed response and Oua concentration? With these questions in mind, we studied the depressive action of Oua on the toad heart contraction *in vitro*.

MATERIALS AND METHODS

The experimental procedures were previously described[2]. In short, 50 toads of either sex weighing $70 \pm s$ 15 g were used in the Straub heart preparation filled with Ringer solution (NaCl 116, KCl 1.2, $CaCl_2$ 1.1, $NaHCO_3$ 2.7 mmol·L^{-1}). The simultaneous recordings of mechanical tension of heart contraction and surface electrocardiogram (ECG) were performed continuously on a 3-channel polygraph. Oua (Sigma) 0.01 — 30 mmol·L^{-1} in Ringer solution was administered by exchanging the solution. Results were expressed as $\bar{x} \pm s$, and the homogeneity of variance of timed responses between various concentration groups was tested by χ^2 method. The [illegible] of timed responses among groups were ana[illegible] F or F' test. $EC_{50} \pm L_{95}$ was calculated by [illegible] method[8], in which L_{95} is the average 95% c[illegible]e limit. Latency and cardioplegia time (CPT, [illegible]tency of cardioplegia) data were nonlinear lea[illegible]are fit, weighted with the square of SE inversely, to the hyperbolic 4-parameter model II; $\hat{Y} = c^{s+1}/(|\ln X - a|)^s + b$, in which $\hat{Y}$ is latency or CPT, X [illegible] concentration, and a, b, c, s are the dosage, [illegible] curvature, and skewness parameter, respectiv[illegible]. The regression significance and goodness of [illegible] analyzed by F or F' test. The half equilibrium [illegible]s fit to linear model or to $\hat{Y} = 0.693/(K_1 X + K_2)$[illegible], in which K_1 and K_2 are association and dissociation rate constants, respectively.

RESULTS

Decrease of contraction tension In 50 heart preparations, the contraction tension was concentration-dependently decreased by Oua 0.01 — 30.0 mmol·L^{-1} (Tab 1), usually with lowering of frequency or ECG arrhythmia (Fig 1). Cardioplegia (100% depression) was seen only at concentrations $\geq$ 1 mmol·L^{-1}. $EC_{50} \pm L_{95}$ was calculated to be 35 ± 8 μmol·L^{-1} from the data of 0.01 — 1.0 mmol·L^{-1}.

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Tab 1. Depressive action of ouabain on toad heart contraction *in vitro*. $\bar{x}\pm s$ (n). [c]$P<0.01$ by F' test, [f]$P<0.01$ by F test.

	Ouabain concentration/mmol·L^{-1}							
	0.01	0.03	0.10	0.30	1.00	3.00	10.0	30.0
% depression of tension (%)[c]	28±22 (5)	37±14 (7)	58±20 (5)	67±12 (7)	99±3 (8)	100±0 (8)	100±0 (5)	100±0 (5)
Peak-reaching time (s)[c]	307±86 (5)	320±175 (7)	440±290 (5)	774±306 (7)	366±231 (8)	249±116 (8)	144±108 (5)	123±85 (5)
Half equilibrium time (s)[f]	148±14 (5)	137±20 (6)	125±23 (6)	112±24 (7)	90±28 (8)	75±36 (7)	56±21 (4)	50±23 (4)
20% depression time (s)[f]	61±7 (5)	53±4 (5)	45±3 (6)	39±7 (6)	26±8 (8)	24±7 (7)	24±9 (6)	20±8 (6)

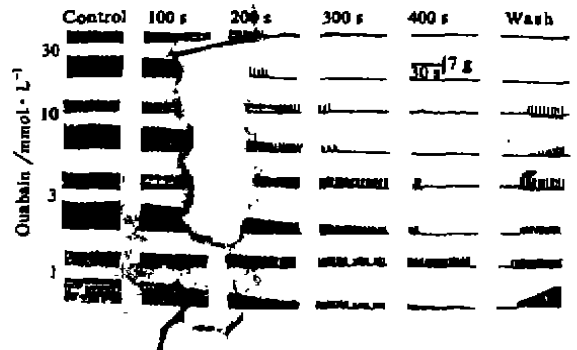

Fig 1. Concentration-dependent toxic action of Oua on the toad heart preparations *in vitro*. Simultaneous recording of surface ECG (upper tracings) and contraction tension (lower tracings). Calibration of 30 s for both ECG and tension recording, 7 g for tension only. Note the slowing of contraction frequency and ECG arrythmia during drug action.

Latency of action Latency of action, defined as the time from exchanging the Oua solution to the beginning of depression of contraction tension, was found to be concentration-dependent at 0.01 — 30 mmol·L^{-1} (Fig 2). The nonlinear least-square fitting equation of the data was:

$$\hat{Y}=25.9492^{7.2757}/(\ln X+23.5906)^{6.2757}+72.7732$$

in which $\hat{Y}$ is the latency, X is the Oua concentration. The dosage parameter a is -23.5906 which means natural logarithm of theoretical threshold concentration of depression. The response parameter b is 72.7732 denoting the theoretical shortest latency. The curvature parameter c (25.9492) reflects the measurable range between b and infinity latency. The skewness parameter s (6.2757) was sensitivity of latency to change of Oua concentration. By F' test, the regression was very significant ($P<0.01$), the goodness-of-fit was satisfactory ($P<0.01$) and

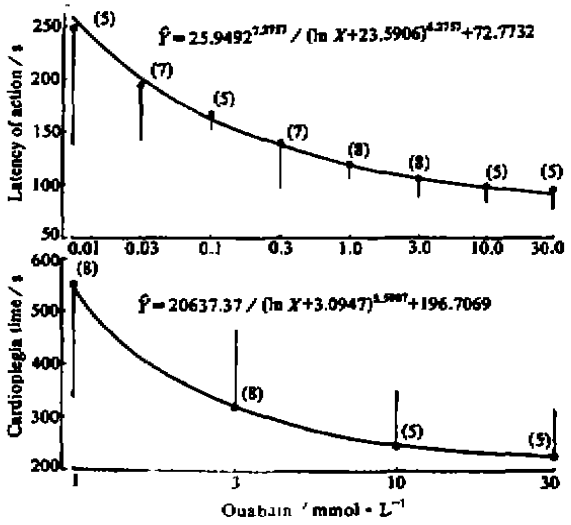

Fig 2. Relations of Oua concentration-latency of depressive action and cardioplegia time on toad heart contraction *in vitro*. (n in parentheses). $\bar{x}\pm s$. F test of $\bar{x}$ for CPT and F' test for latency; $P<0.01$.

the weighted residual square error of fit was insignificant ($P > 0.05$).

Cardioplegia time (CPT) Because of homoscedasticity of variance ($P > 0.05$), F test of $\bar{x}$ among groups showed a very significant difference of CPT (Fig 1, 2). The data were also nonlinear least-square fit to the model II: $a = -3.0947$, $b = 196.7069$, $c = 8.7070$, and $s = 3.5907$ (Fig 2), in which the parameters were similar to those in latency results, but $\hat{Y}$ is CPT and b is the shortest CPT. Similarly, the regression and goodness-of-fit were statistically significant ($P < 0.01$) and residual square error of fit was nonsignificant ($P > 0.05$) by F test.

Peak-reaching time, half equilibrium time, and 20% depression time Due to the heteroscedasticity of variance of peak-reaching time data ($P < 0.01$), F' test was used to analyze the differences of $\bar{x}$ among groups. It was shown that the peak-reaching time was concentration-dependent ($P < 0.01$) and the nonlinear relation was present with the longest time at concentration of 0.3 mmol·L^{-1} (Tab 1).

The half equilibrium time (time to half of maximal depression) and 20% depression time (time to 20% depression of control tension) were negatively proportional to concentration (Tab 1). Although $\hat{Y} = 0.693/(K_1X + K_2)$ was not suitable to fit the half equilibrium time data, a negatively proportional relation to lg concentration was indicated by the linear regression of $\hat{Y} = -13.057 \ln X + 91.4336$ with $r = -0.9952$ ($P < 0.001$) and nonsignificant residual square error of fit ($P > 0.05$). The linear regression of 20% depression time with natural lg of concentration ($\ln X$) was $\hat{Y} = -5.2140 \ln X + 33.3862$ with $r = -0.9643$ ($P < 0.01$).

DISCUSSION

The observed hyperbolic type of TDRR in the speed of appearance (latency or CPT), and concentration-dependent TDRR in the developing rate (peak-reaching time, half equilibrium time and 20% depression time) of Oua depression action on toad heart contraction *in vitro* were consistent with those of our previous studies on other drug actions[2,5,7]. However, the TDRR mechanisms in pharmacokinetics and pharmacodynamics remain unsolved.

The values of parameter a, b, c and s estimated from the latency and CPT data were the representative parameters to characterize Oua action, and also the comparative parameters between drugs and actions. For example, a lower a value (-23.5906) of latency compared with CPT (-3.0947) meant a much lower concentration for depression than nullification of toad heart contraction.

Another interesting finding was the nonlinear relation of peak-reaching time to concentration. It was in contradistinction to the theoretical relation of drug-receptor interaction[8] and also inconsistent with the results of drug actions on rabbit blood pressure[6,7]. The explanations could be: First, the heart preparation *in vitro* was different from a theoretical simple apartment. Second, a qualitative change of response (cardioplegia) was induced at concentrations $\geqslant 1$ mmol · L^{-1}. Third, the intensity of response was the same (cardioplegia) at concentrations $\geqslant 1$ mmol · L^{-1}, but different (28 — 67% depression) at concentrations $\leqslant 0.3$ mmol · L^{-1}. And the negative relation of concentration — 20% depression time strongly supported these view points.

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REFERENCES

1 Wang MY, Sun RY. Review on timed dose-response re-

lationship. *Chin Pharmacol Bull* 1987; 3 : 111—4.
2 Zhang FT, Wang MY. Timed dose-response relationship of toxicological action of ouabain on the toad heart *in vitro*. *Chin Pharmacol Bull* 1993; 9 : In press.
3 Wang MY, Sun RY. An investigation on mathematical models for timed dose-response relationship of drugs. *Bull Sci Technol* 1986; 2 (4) : 33—4.
4 Sun RY, Wang MY. Review and prospect on mathematical toxicology. In: Chinese Pharmacological Society, editor. *Advances in pharmacology* (1986). Beijing: The People's Medical Publishing House, 1987 : 313—26.
5 Wang MY, Sun RY. Mathematical models for hyperbolic type of timed dose-response relationship of drugs. *Acta Pharmacol Sin* 1987; 8 : 481—6.
6 Zheng JQ, Wang BA, Wang MY. Timed dose-response relationship of norepinephrine in raising blood pressure of the rabbits. *J Wannan Med Coll* 1992; 11 : 83—6.
7 Wang MY, Zheng JQ, Wang BA. Timed dose-response relationship analysis of pressor and hypotensive action of drugs. *Acta Pharmacol Sin* 1992; 13 : 501—4.
8 Sun RY. *Quantitative pharmacology*. 1st ed. Beijing: The People's Medical Publishing House, 1987 : 393—452.

哇巴因抑制离体蟾蜍心脏收缩作用的时反应量-效关系[1]

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摘要 对离体蟾蜍心脏的收缩张力-心电同步记录，发现哇巴因（Oua）0.01—30 mmol·L^{-1}抑制收缩作用的张力抑制率，潜伏期，达峰时间，半平衡时间，20%抑制时间及心麻痹时间均呈浓度依赖性（$P<0.01$），且潜伏期和心麻痹时间为双曲线型四参数模型拟合，半平衡时间与对数浓度呈直线关系，表明Oua作用的发生与发展均存在时反应量-效关系。

关键词 哇巴因；心脏；药物剂量-效应关系；心电描记术；时间；回归分析

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Effects of bretylium tosylate on electrophysiologic properties of normal and digitalized papillary muscles of guinea pigs

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ABSTRACT The effects of bretylium tosylate (BT) on the electrophysiologic properties of normal and digitalized papillary muscles isolated from guinea pigs were studied with regular glass microelectrode. BT prolonged effective refractory period (ERP) and action potential duration (APD) of normal papillary muscles. The ERP and APD of papillary muscles were shortened by perfusion with ouabain (Oua) 0.2 μmol·L^{-1}. No recovery was seen in perfusion without drug for 30 min. In digitalized papillary muscles with Oua, ERP, APD_{50}, and APD_{90} were prolonged by BT 120 μmol·L^{-1} from 175±20, 187±20, and 146±21 ms to 222±21, 220±19, and 190±19 ms, respectively. The results demonstrated that BT can prolong ERP and APD of papillary muscles digitalized with Oua.

KEY WORDS papillary muscles; electrophysiology; bretylium tosylate; ouabain

Bretylium tosylate (BT) is an anti-arrhythmic agent of class III. BT prolongs the effective refractory period (ERP) and action potential duration (APD) of isolated Purkinje fibers and ventricular myocardium. BT is effective on various arrhythmias, but controversial results have been reported on those induced by digitalis[1,2]. This paper studied the

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