# COMBINED INOTROPIC EFFECT OF STROPHANTHIN AND CALCIUM ON HEART MUSCLE

I. S. Chekman, N. A. Gorchakova,
V. V. Bratus', and I. S. Mudraya

UDC 615.22:547.918]:582.937].015.2:
615.31:546.41].015.4.616.127

KEY WORDS: strophanthin G; calcium; contractile function of heart muscle.

The use of cardiac glycosides in clinical practice has demonstrated the importance and irreplaceability of this group of drugs for the treatment of heart failure; however, the mechanism of their action remains an unsolved problem [2, 3, 5, 7, 8]. There is no doubt about the potentiating effect of calcium on the action of cardiac steroids on the myocardium. However, data on the role of $Ca^{++}$ in the inotropic action of glycosides are contradictory [9]. The formation of physicochemical complexes of glycosides in solutions has been established and participation of complex formation between cardiac glycosides and calcium in the realization of the primary pharmacologic reaction of this group of drugs has been suggested [4].

The aim of this investigation was to study the physiological action of strophanthin G together with calcium in concentrations corresponding to complex formation, on the myocardium of warm-blooded animals.

## EXPERIMENTAL METHOD

Experiments were carried out on the atrial auricles of 31 guinea pigs weighing 200-300 g. The muscles contracted under the influence of electrical stimulation (square pulses, 10-20% above threshold voltage, duration 5 msec, frequency 2.0 Hz) from an ÉSU-1 electrostimulator in thermostatically controlled (28-29°C) nutrient Tyrode solution of the following composition (in mM): NaCl 118.4, KCl 4.9, $CaCl_2$ 2.5, $NaHCO_3$ 2.5, $MgCl_2$ 1.2, $NaH_2PO_4$ 1.2, glucose 10.0. The force of isometric contractions and its first derivative were recorded on a 6NÉK automatic writer. Steady-state values of parameters of contractility were determined while the muscles were perfused with normal Tyrode solution and with Tyrode solutions containing different concentrations of calcium and strophanthin G. The concentrations of the latter were those giving the highest constant of stability of $Ca^{++}$–strophanthin complexes [1]. In the experiments of series I changes in contractility of the heart muscles were studied on addition of strophanthin and calcium to the Tyrode solution. In the experiments of series II parameters of contractile function of the muscles were studied 120 and 180 sec after the beginning of perfusion with Tyrode solution containing virtually no calcium, but with addition of strophanthin or a combination of strophanthin and calcium in the concentrations used for testing. The time during which the amplitude of contractions fell by 50% during perfusion with the above-mentioned solutions was measured. Crystalline strophanthin G (ouabain, USA) and a 10% solution of calcium chloride in ampuls were used.

## EXPERIMENTAL RESULTS

A dose-dependent inotropic effect of strophanthin was found in these experiments (Fig. 1). If the calcium concentration in the Tyrode solution was increased, instead of a lower inotropic effect a toxic effect of strophanthin was more frequently observed. For instance, with strophanthin in a concentration of $5.10 \times 10^{-6}$ M together with $10.65 \times 10^{-6}$ M of calcium, disturbances of the regular rhythm of contractions of the guinea pig myocardium were observed in 45% of experiments, whereas if strophanthin alone was used, they were observed in 30%. Similar results, indicating weakening of the positive inotropic action of strophanthin with

Department of Pharmacology, Kiev Medical Institute. Laboratory of Experimental Cardiology, N. D. Strazhesko Kiev Research Institute of Cardiology. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 320-323, March, 1985. Original article submitted February 24, 1984.

0007-4888/85/9903-0317$09.50  

TABLE 1. Time Taken to Reduce Force of Contractions of Guinea Pig Myocardium by 50% during Perfusion with Solutions with Reduced Calcium Concentration in the Presence of Strophanthin G and with a Combination of Strophanthin G and Calcium

| Substances tested and their concentration, M | Calcium virtually absent | $\cdot 10^{-8}$ M | $\cdot 10^{-7}$ M | $\cdot 10^{-6}$ M |
|---|---|---|---|---|
| Calcium 10.65 | 62±8 | 78±7 | 81±6 | 120±6*· |
| Strophanthin G 5.10 | — | 66±7 | 97±10 | 115±15* |
| Strophanthin G 5.10+calcium 10.65 | — | 107±10* | 133±10*· | Force of contractions does not fall |

Legend. *P < 0.05 compared with perfusion in virtually calcium-free solution (n = 7).

TABLE 2. Time Course of Parameters of Contractility of Guinea Pig Myocardium during Perfusion with Solutions with Reduced Calcium Concentration and Containing Strophanthin G and a Combination of Strophanthin G with Calcium

| Substances tested and their concentration, M | Initial value | | | After perfusion, % of initial value | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 120 sec | | | 180 sec | | |
| | F, $mN/mm^2$ | $+F'$, $mN/mm^2 \cdot sec^{-1}$ | $-F'$, $mN/mm^2 \cdot sec^{-1}$ | F | $+F'$ | $-F'$ | F | $+F'$ | $-F'$ |
| Calcium 10.65: | | | | | | | | | |
| Calcium virtually absent | 1,65±0,52 | 31,75±8,62 | 22,27±5,59 | 19±4 | 19±4 | 15±5 | 11±3 | 11±3 | 6±2 |
| $\cdot 10^{-8}$ | 2,21±0,2 | 40,02±15,19 | 24,7±8,04 | 19±4 | 17±2 | 12±2 | 9±1 | 8±1 | 7±2 |
| $\cdot 10^{-7}$ | 1,92±0,63 | 34,89±12,54 | 24,7±7,25 | 22±3 | 19±3 | 20±5 | 14±2 | 11±2 | 7±2 |
| $\cdot 10^{-6}$ | 2,06±0,69 | 37,83±14,41 | 28,32±8,62 | 37±3 | 31±4 | 34±7 | 11±2 | 10±1 | 10±2 |
| Strophantin G 5.10 | | | | | | | | | |
| $\cdot 10^{-8}$ | 1,22±0,28 | 25,19±3,33 | 15,39±2,65 | 25±6 | 33±3 | 35±9 | 13±2 | 10±1 | 13±3 |
| $\cdot 10^{-7}$ | 1,21±0,25 | 25,28±6,47 | 17,15±4,9 | 28±5 | 31±3 | 38±3 | 18±1 | 18±3 | 17±4 |
| $\cdot 10^{-6}$ | 1,15±0,28 | 25,48±3,53 | 17,64±3,33 | 47±8 | 52±2 | 72±2 | 19±3 | 26±3 | 23±5 |
| Strophanthin G 5.10+ calcium 10.65 | | | | | | | | | |
| $\cdot 10^{-8}$ | 1,4±0,25 | 28,62±6,47 | 18,23±4,41 | 35±8 | 39±4 | 40±6 | 21±3 | 19±6 | 19±4 |
| $\cdot 10^{-7}$ | 1,23±0,32 | 25,68±7,45 | 16,95±5,19 | 51±3 | 52±3 | 47±4 | 24±6 | 24±6 | 30±4 |
| $\cdot 10^{-6}$ | 0,99±0,14 | 15,29±1,18 | 9,21±1,57 | 61±3 | 60±2 | 79±3 | 91±15 | 91±15 | 85±12 |

Legend. F) maximal force of isometric contraction relative to area of cross section, calculated by dividing weight of muscle by its length, taking its specific gravity as 1; +F') maximal rate of contraction; −F') maximal rate of relaxation.

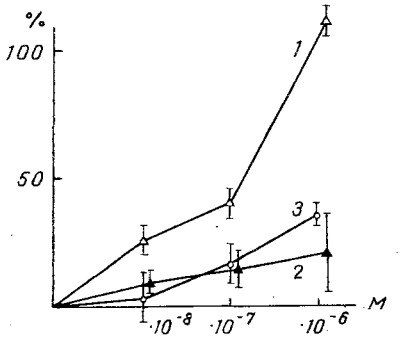


Fig. 1. Dependence of maximal isometric contraction of heart muscle (abscissa) on concentrations of strophanthin G, calcium, and combinations of calcium with strophanthin G in Tyrode solution (ordinate). 1) Strophanthin G (5.10); 2) strophanthin G (5.10) and calcium (10.65); 3) calcium (10.65); 28-29°C, 2.0 Hz.

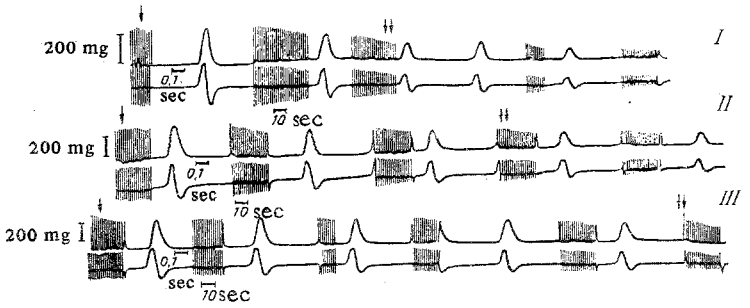


Fig. 2. Contractile function of guinea pig myocardium during perfusion with Tyrode solution containing virtually no calcium (I), in the same solution containing strophanthin G ($5.10 \times 10^{-7}$ M) (II), and containing $5.10 \times 10^{-7}$ M strophanthin G together with $10.65 \times 10^{-7}$ M calcium (III). Arrow indicates time of change from perfusion with ordinary Tyrode solution to perfusion with abovementioned solutions; two arrows indicate time when amplitude of contractions was reduced by 50%. Experiment on May 24, 1983.

an increase in extracellular calcium concentration have been published previously [6]. The effect of particular combinations of strophanthin G and calcium on contractility of the heart muscle could not be demonstrated by these experiments, for calcium concentrations in the Tyrode solution used to perfuse the isolated heart muscles were several orders of magnitude higher ($2.5 \cdot 10^{-3}$ M) than those required to form complexes with strophanthin, as shown by the results of physicochemical investigations [1].

In the experiments of series II the dynamics of myocardial contractility was studied during perfusion with Tyrode solution virtually free from calcium and with the addition of strophanthin G and calcium in concentrations necessary for complex formation. Traces of one experiment when heart muscles, after contracting in ordinary Tyrode solution, began to be perfused with calcium-free solution, are illustrated in Fig. 2. Under these circumstances, naturally, there was a rapid decline in contractility of the muscles. As Table 1 shows, with a change to perfusion with virtually calcium-free solution the amplitude of contraction fell by 50% on average in 62 sec. Addition of calcium to the original solution lengthened this time.

Addition of strophanthin G alone to virtually calcium-free Tyrode solution also led to some lengthening of the time taken for contractility to decline, but in the presence of calcium in a concentration of $5.1 \times 10^{-6}$ M this time was significantly increased. This was probably due to displacement of membrane-bound calcium by strophanthin, an increase in the quantity of mobile calcium, i.e., capable of taking part in the act of contraction, and elevation of the intracellular calcium level [7, 8].

The results in Table 2, showing the time course of contractile function of the heart muscles during perfusion with calcium-free solutions, demonstrate that parameters of myocardial contractility fell by a lesser degree compared with those on the change to perfusion with calcium-free solution on the addition of calcium, and even less, on the addition of strophanthin G.

A combination of strophanthin G with calcium, in concentrations as low as $5.10 \times 10^{-8}$ and $10.65 \times 10^{-8}$ M respectively, led to significant lengthening of the time taken by the force of contractions to fall, by 42 ± 5 sec (analysis by the differences method), whereas neither strophanthin nor calcium separately, in the concentrations given above, changed that time significantly (Table 1). An even more marked effect of delayed depression of contractility of the heart muscles after perfusion with virtually calcium-free solution on the addition of a combination of $5.10 \times 10^{-7}$ M strophanthin and $10.65 \times 10^{-7}$ M calcium was observed: The time was increased by 83 ± 13 sec. With strophanthin and calcium present in concentrations of $5.10 \times 10^{-6}$ and $10.65 \times 10^{-6}$ M respectively, however, the force of contractions was virtually unchanged (Table 2). It can be tentatively suggested that it is this

combination of strophanthin G with calcium that is optimal for manifestation of a cardiotonic effect, possibly as a result of complex formation in the solution and stabilization of the glycoside molecule in the cis position.

The weaker effect of a combination of strophanthin and calcium compared with that of strophanthin alone, added to the ordinary Tyrode solution, may be due to greater intracellular accumulation of calcium, interference with its removal at binding sites, and disturbance of electromechanical coupling processes [10].

The change in contractility of the heart muscles during perfusion with calcium-free solution takes place as the result of a reduction (because of washing out) of the quantity of calcium bound with the sarcolemma, whereas the concentration of nonmetabolic calcium remains constant [9]. Addition of strophanthin to calcium-free Tyrode solution probably increases the quantity of calcium capable of participating in the act of contraction. A combination of strophanthin with low concentrations of calcium is an optimal combination of these substances (possibly as a result of complex formation and conformational changes to the cis form of the glycoside), facilitating interaction between the cardiac steroid and sarcolemmal $Ca^{++}$-binding sites, and facilitating realization of the inotropic effect. Administration of strophanthin together with calcium preparations with established ratios between the components can be recommended in clinical practice.

LITERATURE CITED


1. N. A. Gorchakova, L. I. Budarin, R. V. Suchkova, et al., Farmatsevt. Zh., No. 4, 53 (1978).
2. F. Z. Meerson, Kardiologiya, No. 9, 143 (1977).
3. I. S. Chekman, Vest. Akad. Med. Nauk SSSR, No. 5, 29 (1982).
4. I. S. Chekman, L. I. Budarin, N. A. Gorchakova, et al., Farmakol. Toksikol., No. 2, 57 (1983).
5. A. I. Cherkes, Vrach. Delo, No. 13, 1101 (1949).
6. J. Halliday and S. E. Harding, Brit. J. Pharmacol., 66, 1 (1979).
7. P. C. Maitland, S. V. Lamont, and G. J. Barritt, Biochem. Pharmacol., 31, 2471 (1982).
8. W. Nayler and E. Noack, in: Cardiac Glycosides, Part I. Experimental Pharmacology, ed. K. Greeff, Berlin (1981), pp. 407-436.
9. P. A. Wanderson, R. Manring, J. R. Sommer, et al., J. Molec. Cell. Cardiol., 8, 123 (1976).
10. K. C. Wong, S. Sullivan, and D. Westone, Fed. Proc., 33, No. 3, 503 (1974).


# PHARMACOETHOLOGIC ANALYSIS OF THE ACTION OF SOME β-CARBOLINES


V. P. Poshivalov



UDC 616.89-008.444.9-092.9-02:615.214.547.562

KEY WORDS: pharmacoethology; β-carbolines; benzodiazepines; aggression; intraspecific behavior.


Compounds of the β-carboline group are physiologically active substances with a wide spectrum of pharmacologic action: They simulate effects of serotonin [6], counteract effects of benzodiazepines (BDZ) [7], participate in the formation of dependence on ethanol [1, 3], modify behavior [4], and can induce tremor [3, 6]. The ability of β-carbolines to regulate intraspecific behavior and, in particular, aggression, defense, and sociability, has not been studied previously.

The aim of this investigation was to study pharmacoethologic spectra of the action of β-carbolines and their influence on intraspecific aggression, sociability, and individual behavior.

---


Department of Pharmacology, Academician I. P. Pavlov First Leningrad Medical Institute.


(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 323-325, March, 1985. Original article submitted January 13, 1984.




0007-4888/85/9903-0320$09.50