EXPERIMENTAL CARDIO-DEPRESSANT EFFECTS OF CLONIXIN*

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Abstract—1. The cardiovascular effects of CLX were studied.

2. CLX induced hypotension, bradycardia, negative chronotropism and negative inotropism.

3. Electrophysiological studies showed a decrease of sinus venosus discharge frequency. The action potential configuration was changed: the overshoot amplitude and $(dV/dt)_{max}$ were reduced and duration increased.

4. CLX at higher concentrations displaced the maximum diastolic potential and phase 4 slow diastolic depolarization was lengthened.

5. The above findings could be explained by a depressant action of CLX on the electrical activity of the pacemaker cells, possibly by a modification of the slow calcium currents.

INTRODUCTION

Clonixin (CLX) is a drug that displays anti-inflammatory analgesic and antipyretic activity in rats and monkeys (Watnick et al., 1971; Ciofalo et al., 1972). In humans it has been proposed as analgesic for oral and parenteral use (Finch and De Kornfeld, 1971; Paredes et al., 1986) with certain advantages over others non-steroidal anti-inflammatory agents because of its lack of activity over platelet aggregation (Arkel et al., 1976) and a good anti-inflammatory/ulcerogenic ratio (Watnick et al., 1968, 1971).

The study of the effects CLX in the isolated vas deferens of the rat under transmural neurogenic stimulation (TNS) demonstrated that CLX produced a significant reduction of TNS induced muscular twitch, and potentiated the alpha$_2$-NE response that this organ exhibits (Bustamante et al., 1988). These results suggest that CLX may block NE release through a similar mechanism of action as calcium channel blockers like verapamil, diltiazem and nifedipine (Arqueros and Daniels, 1978; Godfraind et al., 1986).

In addition, a recent comparative study of the analgesic effect between CLX, nifedipine and morphine (Bustamante et al., 1989) could suggest that calcium channel blockers not only are useful in angina as vasodilators, but they also have analgesic properties of their own. Recently, a relation between the opioid receptor and the calcium channel has been reported; this correlation is evidenced by a decreased voltage dependent calcium conductance and changes in the intracellular calcium levels (MacDonald and Herz, 1986; Pillai and Ross, 1986; North, 1986; Gross and MacDonald, 1987; Kavaliers and Ossenkopp,

1987) these findings agree with old evidence that link morphine analgesia and calcium (Kakunaga et al., 1966; Harris et al., 1975; Chapman and Way, 1982).

Previous findings in this laboratory, have demonstrated that CLX induces changes in the EEG and in the cardiovascular system of the rat, like bradycardia and hypotension. Other results indicate that CLX blocks NE release in isolated vas deferens, both findings suggested the possibility that it could exert its action through the blockade of calcium currents. It was therefore designed to study the cardiovascular effects of CLX at three different levels: (i) in an in vivo preparation mean blood pressure, heart and respiratory rate were studied; (ii) in an in vitro model of isolated atria of the rat, inotropic and chronotropic action of CLX were studied and (iii) in frog sinus venosus electrophysiological studies in two populations of excitable cells were performed.

MATERIALS AND METHODS

Cardio-respiratory experiments

Adult Wistar rats weighing 200–300 g were used throughout this work. The animals were anesthetized with urethane (1 g/kg i.p.) and then the femoral vein was dissected and cannulated for i.v. injections. Also in the trachea a stainless steel cannula was installed and connected to an A. Fleisch PT 5A pneumotachograph to record respiratory frequency (RF) and the right carotid artery connected to a Statham P25 DC transducer for recording mean blood pressure (MBP). The heart rate (HR) was calculated from D_{II} or D_{III} ECG derivatives. After 15 min of stabilization each rat was injected i.v. with one dose of the drug during 1 min and injection volume was kept constant (1 ml/kg). The MBP and RF were measured and compared with basal 3 min after drug injection. Besides, HR is shown at the end of the first minute, since this parameter was found to be significantly different from basal.

Isolated atria preparations

Animals were sacrificed by a cervical dislocation. The heart was quickly dissected and placed immediately in a

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preoxygenated Tyrode solution (Miranda *et al.*, 1979) where atria were removed and mounted in a 20 ml bath filled with Tyrode solution at $30°C$ and oxygenated with a $O_2:CO_2$ (95:5%) mixture. Tension was registered using a Grass FT-03 transducer connected to a Grass polygraph. The left atrium was stimulated by two silver electrodes with square pulses (4 msec, 15 V and 4 Hz) and the right was left spontaneously beating.

After a stabilization period of 1 hr under a load of 0.5 g, using a non-cumulative dosage schedule, one isometric contractile dose response curve for CLX was obtained. Effects were measured 5 min after the drug was added to the bath. Results are expressed as percent from basal.

Electrophysiological experiments

Frogs from the *Caudiverbera caudiverbera* species weighing between 200–350 g were used throughout these experiments. Animals were sacrificed by spinal cord transection. The heart was dissected and transferred to an oxygenated Ringer solution (Morales *et al.*, 1988). The sinus venosus was isolated by microdissection and fixed in a 12 ml organ bath superfused continuously (3 ml/min) with oxygenated (100%) Ringer solution at room temperature (20°C). Action potentials from primary and transitional cells were recorded differentially using glass microelectrodes filled with 3 M KCl and a reference electrode Ag/AgCl–agar Ringer (tip resistance: 15–30 MΩ). The recording system is composed of an electrometer amplifier with capacity compensation and a time constant of 40 μsec. The output is displayed in a dual beam oscilloscope and also displayed, analyzed and recorded using an IBM compatible XT (640 kbyte) computer (Hercules[x] high resolution graphics card) with a digital to analog converter and *ad hoc* software devised to calculate the action potential parameters.

Drugs assayed were added to the Ringer solution and recordings were taken 30 and 60 min after drug superfusion. Each preparation was exposed to only one drug concentration.

Data are expressed as mean ± SEM. Significance of the differences were analyzed according to Student's *t*-test. The level of probability accepted as significant was 0.05.

Clonixin was supplied by Pharma Investi Laboratory, Santiago, Chile.

RESULTS

Cardio-respiratory experiments

The CLX dose range used was 10–120 mg/kg and the i.v. injection produced a rapid dose-dependent hypotension with and ED_{50} of 38 mg/kg (Fig. 1). The maximum effect was obtained with 100 mg/kg and elicited a 35% decrease in MBP which returned to baseline value (135 ± 5 mmHg, $n = 60$) between 5–10 min.

The effect of CLX 10–120 mg/kg of RF did not differ from control values. The HR decreased during the first min in a dose-dependent fashion, as shown in Fig. 2, after this time the HR returned to normal values. With doses higher than 120 mg/kg the animals exhibited intense hypotension, bradycardia, bradypnea and died.

Isolated atria preparations

CLX (10^{-6}–10^{-3} M) induced a concentration dependent negative inotropic effect in electrically driven left atria and a negative chronotropic effect on spontaneously beating right atria (Fig. 3). The IC_{50} for both preparations was similar (0.20 and 0.17 mM respectively).
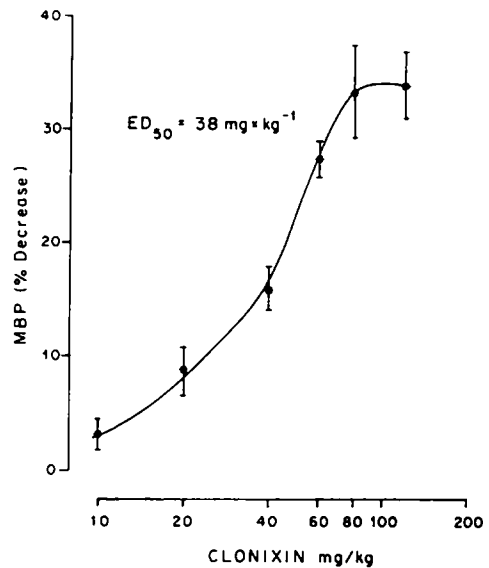

Fig. 1. Mean blood pressure (MBP) dose effect curve of CLX in urethane anesthetized rats. Each point represents the mean ± SEM of 6 experiments.

Electrophysiological experiments

Effect of CLX on sinus rate. Preparations of the sinus venosus beating spontaneously were superfused with CLX added to the Ringer solution in concentrations which ranged from 1×10^{-6} to 5×10^{-6} M. The drug decreased sinus rate in all experiments; with 2×10^{-6} M CLX sinus rate was decreased 14% within 60 min of exposure and with 5×10^{-6} M the effect reached 20%.

Effect on primary pacemaker cells. As summarized in Table 1, CLX (2×10^{-6} M) did not change the maximum diastolic potential (MDP) nor threshold potential (TP), but reduced the amplitude (APA) (13.6%), overshoot (OS) (60.2%) of primary pacemaker cells. Moreover, the drug prolonged the action
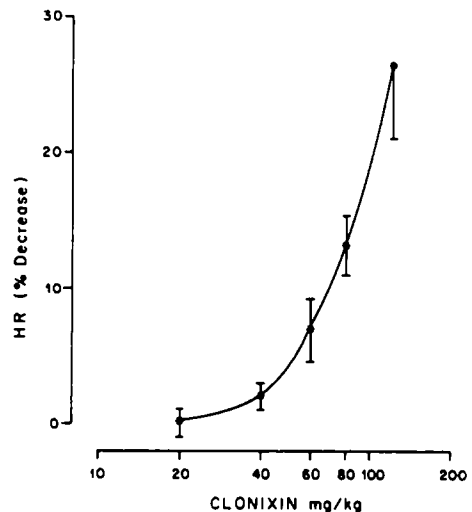

Fig. 2. Heart rate (HR) dose effect curve of CLX in urethane anesthetized rat. Each point represents the mean ± SEM of 6 experiments.
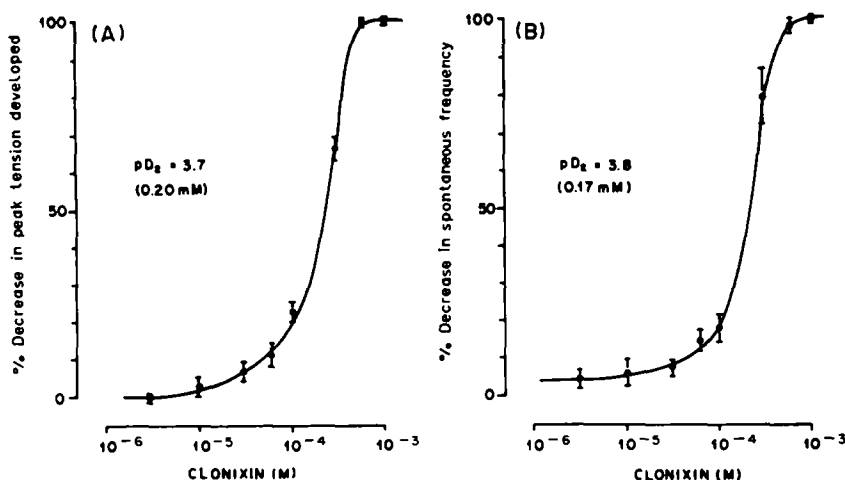
Fig. 3. Inotropic (A) and chronotropic (B) effects of CLX in isolated atria of rats. Each point represents the mean ± SEM of 6 experiments.

Table 1. Electrophysiological effects of CLX on primary cells of the frog sinus venosus

	Control	CLX 2 μM
TP (mV)	44.4 ± 0.9	46.8 ± 1.9
MDP (mV)	59.0 ± 0.4	57.3 ± 2.1
APA (mV)	62.5 ± 1.3	54.0 ± 2.3*
OS (mV)	18.1 ± 1.4	7.2 ± 1.3**
APD$_{60}$ (msec)	634.7 ± 21.4	713.5 ± 12.9*
Rate (beats/min)	29.4 ± 0.8	25.4 ± 0.2**
n	11	9

Each value is the mean ± SEM of the number of experiments indicated by n. *$P < 0.01$; **$P < 0.001$.

potential duration (APD$_{60}$) about 12%, also the $(dV/dt)_{max}$ was decreased 50%. These results were obtained 20–60 min after drug exposure and represent a steady state effect. At the end of this period (60 min), a greater depression of the primary cell action potential was observed (Fig. 4). The blocking effect of CLX 2×10^{-6} M on primary cells after 75 min is illustrated in Fig. 4. Total amplitude was remarkably reduced, threshold and maximum diastolic potential were displaced to a less negative value (Fig. 4). Action potential of transitional pacemaker cells (typified by Hernández et al., 1987) registered in the vicinity of primary cells were not modified by the drug.

When the preparation was superfused with CLX 5×10^{-6} M it was impossible to register action potentials from primary cells, which were easily recorded before adding the drug. Transitional cells were partially blocked, phase 4 slow diastolic depolarization was lengthened and overshoot was the parameter most affected (Fig. 5).

DISCUSSION

The results obtained with CLX were hypotension, bradycardia, negative chronotropism and negative inotropism. These cardiodepressant effects were evidenced by electrophysiological studies characterized by decreasing sinus venosus discharge frequency. Moreover, the action potential configuration was changed; the overshoot amplitude and $(dV/dt)_{max}$ were reduced, and the action potential duration was increased. Furthermore, CLX at higher concentrations displaced to a less negative value the maximum diastolic potential. In addition, phase 4 slow diastolic depolarization was lengthened.

The effect of CLX on the action potential of primary pacemaker cells was evidenced as a decrease in the rate of rise of phase 0 and could be explained by a modification of the slow calcium currents. These results are in agreement with the verapamil induced action potential modification obtained by Morales et al. (1988), since this phase of primary cells have been found to be calcium current dependent.

The depressed electrical activity of the primary cells is revealed by the lengthening of the fire-rate of transitional cells (see Fig. 5). It is not possible to discard that the reason for this negative chronotropic effect of CLX, may be due to a direct action of the drug on the currents that compose the phase 4 of the slow diastolic depolarization. It is known, that the last third of phase 4, is originated by the slow current of calcium (Noble, 1984).

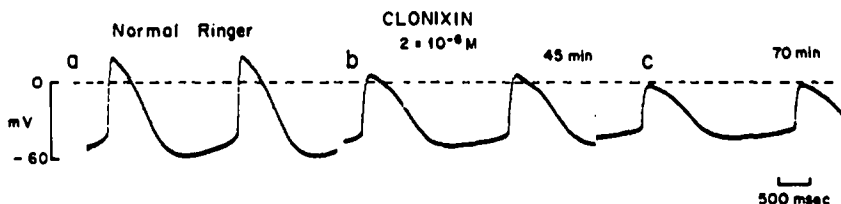The negative chronotropic effect obtained in isolated atria and bradycardia in rats, could be

Fig. 4. Effect of CLX on the action potential of a primary cell from sinus venosus spontaneously beating. (a) control; (b) 45 min after CLX and (c) 70 min after CLX.
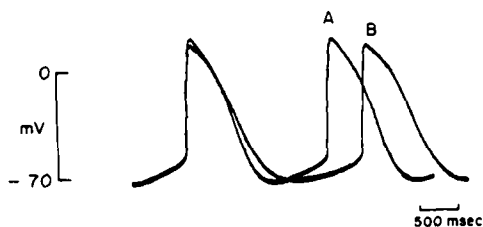
Fig. 5. Superimposed action potentials of frog sinus venosus transitional pacemaker cells showing the CLX effect 45 min after drug exposure.

explained through a direct depressant action of CLX on the pacemaker cells.

Taking collectively, the above findings could be interpreted by a CLX-depressant action of the cardiac function. All these results seem to be in accord with the assumption that the depressant action of CLX is chiefly exerted on the electrical activity of the pacemaker cells; possibly through a modification of the slow current of calcium due to a direct action of CLX or an indirect action through acetylcholine or Na^+-Ca^{2+} exchanger blockade.

Experiments are in progress, with a more precise design to obtain further and detailed information about the pharmacological profile of CLX.

REFERENCES

Arkel Y. S., Schrogie J. J. and Williams R. (1976) Effect of clonixin and aspirin on platelet aggregation in human volunteers. *J. clin. Pharmac.* 16, 30–33.

Arqueros L. and Daniels A. J. (1978) Analysis of the inhibitory effect of verapamil on adrenal medullary secretion. *Life Sci.* 23, 2415–2422.

Bustamante D., Miranda H. F., Paeile C. and Wolstenholme W. W. (1988) Effects of clonixin, a non steroidal anti-inflammatory analgesic, on the neurotransmission of the vas deferens of the rat. *Gen. Pharmac.* 19, 755–758.

Bustamante D., Miranda H. F., Pelissier T. and Paeile C. (1989) Analgesic action of clonixin, nifedipine and morphine using the formalin test. *Gen. Pharmac.* 20, 319–322.

Chapman D. B. and Way E. L. (1982) Modification of endorphin/enkephalin analgesia and stress induced analgesia by divalent ions, a cation chelator and an ionophore. *Br. J. Pharmac.* 75, 389–396.

Ciofalo V. B., Patel J. and Taber R. I. (1972) Antinociceptive activity of clonixin in rhesus monkeys. *Jpn. J. Pharmac.* 22, 749–753.

Finch J. and De Kornfeld T. (1971) Clonixin: a clinical evaluation of a new oral analgesic. *J. clin. Pharmac.* 11, 371–377.

Godfraind T., Miller R. and Wibo M. (1986) Calcium antagonism and calcium entry blockade. *Pharmac. Rev.* 38, 321–416.

Gross R. A. and MacDonald R. L. (1987) Dynorphin A selectively reduces a large transient (N-type) calcium current of mouse dorsal root ganglion neurons in cell culture. *Proc. natn. Acad. Sci. U.S.A.* 84, 5469–5473.

Harris R. A., Loh H. H. and Way E. L. (1975) Effects of divalent cations, calcium chelators and ionophore on morphine analgesia and tolerance. *J. Pharmac. exp. Ther.* 195, 488–498.

Hernández D., Guerrero S. and Morales M. (1987) Electrophysiological characteristics of cardiac pacemaker cells of the frog *Caudiverbera caudiverbera. Comp. Biochem. Physiol.* 87A, 649–656.

Kakunaga T., Kaneto H. and Hano K. (1966) Pharmacological studies on analgesics. VII. Significance of the calcium ion in morphine analgesia. *J. Pharmac. exp. Ther.* 153, 134–141.

Kavaliers M. and Ossenkoop K. P. (1987) Calcium channel involvement in magnetic field inhibition of morphine-induced analgesia. *Naunyn-Schmiedebergs Arch. Pharmac.* 336, 308–315.

MacDonald R. L. and Herz W. A. (1986) Dynorphin A decreases voltage-dependent calcium conductance of mouse dorsal root ganglion neurones. *J. Physiol., Lond.* 377, 237–249.

Miranda H., Huidobro F. and Huidobro J. P. (1979) Evidence for morphine and morphine-like alkaloid response resistant to naloxone blockade in the rat vas deferens. *Life Sci.* 24, 1511–1518.

Morales M., Hernandez D., Ladron de Guevara R. and Guerrero S. (1988) Electrophysiological effects of verapamil on primary and transitional pacemaker cells of the frog heart. *Gen. Pharmac.* 19, 425–430.

Noble D. (1984) The surprising heart: a review of recent progress in cardiac electrophysiology. *J. Physiol., Lond.* 353, 1–50.

North R. A. (1986) Opioid receptor types and membrane ion channels. *TINS* 9, 114–117.

Paredes H., Solis R., Palacios J., Rocha M., Montecinos A. M., Ramos H. and Hernandez A. (1986) The use of intravenous lysine clonixinate for rapid pain relief: an open clinical study. *Current Ther. Res.* 40, 86–91.

Pillai N. P. and Ross D. H. (1986) Interactions of kappa agonists with Ca^{2+} channel antagonists in the modulation of hypothermia. *Eur. J. Pharmac.* 132, 237–244.

Watnick A. S., Taber R. I. and Tabachnick I. I. A. (1968) Pharmacological properties of 2-(2′-methyl-3′-chloro)-anilino-nicotinic acid (SCH 10304) an antiinflammatory agent with analgesic and anti-pyretic action. *Fedn Proc.* 27, 533.

Watnick A. S., Taber R. I. and Tabachnick I. I. A. (1971) Antiinflammatory and analgesic properties of clonixin [2-(2′-methyl-3′-chloro)-anilino-nicotinic acid]. *Archs int. Pharmacodyn.* 190, 78–90.