Pharmacological Properties of Cardiotoxin Isolated from Formosan Cobra Venom*

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Summary. Lyophilized venom of Naja naja atra was fractionated on column of CM-Sephadex C-50 into 12 fractions by gradient elution with ammonium acetate buffer at pH 5–7. Among them 5 fractions (V–IX) were found to be neurotoxic and 3 (X–XII) to be cardiotoxic. Intraperitoneal LD₅₀ in mice was 0.074 µg/g for the major neurotoxic component (Fr. VIII) and 1.48 µg/g for the major cardiotoxic one (Fr. XII). The latter, named cardiotoxin, caused contracture followed by paralysis of the chick biventer cervicis muscle, the frog sartorius muscle and the rat diaphragm, in consequence of irreversible depolarization of the cell membrane. In the absence of calcium no contracture was produced, although the depolarizing effect remained unchanged. Cardiotoxin caused systolic arrest of the isolated frog heart and the rat atrium, probably by the same mechanism. It also caused a contraction of the guinea-pig ileum, which was largely antagonized by atropine but not by hexamethonium or pyribenzamine. In the presence of cardiotoxin the responses of the ileum to nicotine and to submaximal transmural stimulation were first enhanced and then depressed. Cardiotoxin caused vasoconstriction of the rabbit ear vessels, which was largely antagonized by phenoxybenzamine. It also produced local irritation of the rabbit conjunctiva and the rat hind paw. In cats, cardiotoxin caused a fall in the systolic pressure much more than the diastolic pressure, accompanied by various ECG changes, such as P-R interval prolongation, decreased amplitude of QRS, S-T and T changes, ventricular premature beats, complete A-V block, idioventricular rhythm etc. It is concluded that cardiotoxin acts on various kinds of cells in the animal body, causing irreversible depolarization of the cell membrane and consequently impairing its functions.

Key-Words: Cobra venom — Cardiotoxin — Muscle — Cell membrane—Depolarization — Cardiovascular system—ECG.

Schlüsselwörter: Kobragift — Cardiotoxin — Muskel — Zellmembran — Depolarisation — Zirkulation—EKG.

Although the primary cause of death from cobra venom has been shown to be peripheral respiratory paralysis in many species of animals (Kellyway, Cherry and Williams, 1932; Lee and Peng, 1961; Vick, Ciuchta and Polley, 1965), the venom also produces profound cardio-

vascular changes. When animals envenomed with larger doses are maintained by artificial respiration they finally die of circulatory collapse. Several active components such as neurotoxin, cardiotoxin, phospholipase A, and some proteins having other enzymatic activities have been separated from cobra venom (see SLOTTA, 1955; MELDRUM, 1965; BOQUET, 1966). However, it has not been established as to which component(s) or to what extent these components are responsible for the cardiovascular effects caused by crude cobra venom. While cobra neurotoxin has been isolated in crystalline form (YANG, 1965) and the mode of its neuromuscular blocking action has been studied at length (CHANG and LEE, 1966; SUN, CHANG and LEE, 1967), "cardiotoxin" isolated by SARKAR (1947a) has been shown not to be a single protein (RAUDONAT and HOLLER, 1958) and the mode of its action has not been fully elucidated.

In the present investigation, a chemically homogeneous cardiotoxin has been isolated from the Formosan cobra venom by CM-Sephadex column chromatography and its pharmacological properties have been studied in order to shed some light on its mode of action. A preliminary account of this work has already been published elsewhere (LEE, CHANG, CHIU, TSENG and LEE, 1966).

Materials and Methods

**Venom.** The venom of *Naja naja atra* used in this study was freshly collected and lyophilized in this laboratory and stored in dry state in a vacuum desiccator.

**Column Chromatography.** Fractionation of the venom was proceeded according to the method of LO, CHEN and LEE (1966) with minor modifications. CM-Sephadex C-50 was equilibrated with 0.005 M ammonium acetate buffer, pH 5.0 and then packed into a column of 1.6 × 80 cm at 4°C. The gradient was established by adding 0.9 M ammonium acetate buffer, pH 7.0, into a mixing flask containing 450 ml of 0.005 M ammonium acetate buffer, pH 5.0. The flow rate was adjusted to 10 ml/hr. Eluates in the volume of 3 ml were collected for each tube. The elution pattern was followed by reading the absorption at 280 nm with Beckman D.U. spectrophotometer. The eluates corresponding to the same peak were pooled and lyophilized for subsequent study.

**Toxicity in Mice.** Toxicity of each fraction was assayed in mice (NIH strain) weighing 15--20 g by intraperitoneal injection. The concentrations were so adjusted that the selected doses were contained in 0.1--0.2 ml saline per 10 g body weight of mice. For the major toxic fractions subcutaneous toxicity was also assayed. LD₅₀ was computed according to the method of LITCHFIELD and WILCOXON (1949).

**Isolated Frog’s Heart.** The frog (*Rana tigerina*) heart was isolated for perfusion according to the method of Straub. The Ringer’s solution contained (mM): NaCl, 111.2; KCl 2.0; CaCl₂ 1.8 and NaHCO₃ 6.0. All experiments were performed at the room temperature (20--24°C).

**Isolated Rat Atrial Preparation.** The rat atrial preparation was prepared by the method described by BURN (1952) and suspended in 25 ml of well-oxygenated Lock’s solution (29°C), in which the amount of glucose was doubled (2 g/l). The spontaneous contractions of the auricle were recorded on a smoked drum.