ACTION OF THE CRUDE EXTRACT OF CHICOREUS RAMOSUS HYPOBRANCHIAL GLAND ON THE ISOLATED FROG HEART AND RABBIT INTESTINE

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INTRODUCTION

The functions of hypobranchial glands of the muricid gastropod have attracted the interest of zoologists for many years, because they secrete a chromogen which gets oxidized in the presence of light and air, and turns to a dye of intense purple. That dye was well known to the ancient world as Tyrian purple. It was Dubois (1909) who first showed that extracts of these glands besides containing the purple chromogen, also are extremely toxic to warm- and cold-blooded animals, causing muscular paralysis. Vincent & Jullien (1938) drew attention to the high acetylcholine equivalent of these glands and the possible relationship of this factor to their toxicity, a connection which was clarified by the discovery of urocanylcholine, which in addition to possessing certain properties in common with acetylcholine, also is an effective neuromuscular blocking agent (Ersramer, 1953). Whittaker (1960) isolated the pharmacologically active choline esters in marine gastropods. In clinical trials, this toxic choline compound named "murexine" resembles senecioylcholine (Whittaker, 1960). Murexine induces a neuromuscular blocking action, which has about one-fifth of the potency of succinylcholine and, like succinylcholine, murexine has been proposed as an alternate muscular relaxant in clinical use (De Blasi and Leone, 1955; Ciocatto et al., 1956). Therefore the present study was designed to carry out some pharmacological experiments on isolated muscle preparations of frog heart and rabbit intestine to assess their qualitative response to the crude extract (drug) of hypobranchial glands of C. ramosus.

MATERIALS AND METHODS

Extraction of crude venom

The hypobranchial glands of C. ramosus were dissected aseptically and a crude extract of the glandular secretions and contents was prepared following the method adopted by Wallis (1982). The glands were ground with glass pestle in a glass mortar in a physiological saline solution (8.5% NaCl). Then it was centrifuged at 3000 rpm for 5 min and the supernatants collected. The supernatant was dialysed, lyophilized, and finally the crystalized crude extract (drug) was obtained.

Isolated preparation of frog heart

A frog was pithed by destroying its central nervous system. The heart was dissected and the pericardium was removed without injuring the inferior vena cava. Venous cannula was inserted in the aorta and the vena cava was tied with a thread with the cannula. A Marriotte bottle containing Ringer’s solution was connected to the cannula. The drugs were introduced into the heart through the cannula. A needle was attached through the apex of the ventricle to a liver and the heart beats were recorded on a slow moving drum of a kymograph. The blocker used was propranolol (1:100 W/V).

Constituents of Ringer’s solution: NaCl (9.0 g), KCl (0.42 g), CaCl₂ (0.24 g, unhydrous salt), NaH CO₃ (0.5 g), Glucose (1.0 g), Distilled water (1.4 litres).
Figure 1. Action of hypobranchial gland extract of *Chicoreus ramosus* on frog heart (H.E. - Hypobranchial gland extract (10 mg ml⁻¹); PRO - Propranolol; N - Normal)

Figure 2. Action of hypobranchial gland extract of *Chicoreus ramosus* on rabbit intestine (H.E. - Hypobranchial gland extract (10 mg ml⁻¹); Ach - Acetylcholine (20 μm); W - Wash; H - Normal Peristaltic movement)

**Isolated preparation of rabbit intestine:** The jejunum of the rabbit’s intestine was removed along with its mesentery from a freshly killed rabbit. It was kept moist with Tyrode’s solution during further dissection. A segment of the jejunum was selected. With its accompanying section of mesentery it was placed on a thermostat chamber containing Tyrode’s solution. One end of the jejunum was attached to the aerating tube with a fine thread, and was tied to the apex of the mesentery. The other end was tied to a lever. The bath filled with Tyrode’s solution was maintained at 37°C and the drugs were introduced into this chamber. The peristaltic movement were recorded on a slow moving drum of a kymograph. Acetylcholine (2 μg dose) was used as the stimulant.

**Constituents of Tyrode’s solution:** NaCl (6.9 g), KCl (0.2 g), CaCl₂ (0.2 g), MgCl₂ (0.10 g), NaHCO₃ (1.0 g), NaOH₂PO₄ (0.05 g), Glucose (1.0 g), Distilled water (1 litre).

**RESULTS AND DISCUSSION**

The study on isolated muscle preparations was designed to show how the action of the test drug may be modified by adding other drugs. The action
depends on the specificity of the blockers (e.g. propranolol) to block specific receptors (e.g. adrenergic).

When the crude extract preparation of hypobranchial glands of C. ramosus was tested against the frog heart, a dose dependent stimulant action was produced. The blocker propranolol (2 µg dose level), was used to ascertain whether the extract was acting through adrenergic receptors. The action of the stimulant was not blocked by propranolol, which leads to the presumption that the venom may act directly on the heart and not through adrenergic receptors. The sample had been dialysed so the influence of calcium in the extract can be ruled out. The active principle present in the sample might be a directly acting cardiac stimulant (Fig.1), which may be utilized in congestic cardiac failure during heart surgery.

The experiment with the rabbit intestine showed that the hypobranchial gland extract reduced the peristaltic movement of the intestine and in no way blocks the action of acetylcholine, nor does it act as an antagonist or potentiate the action of acetylcholine. From the study it can be assessed that the extract may contain a succinylcholine type of principle (De Blasi and Leone, 1955; Ciocatto et al., 1956) because succinylcholine has partially to do with agonistic and antagonistic action (Fig.2).

Further studies are in progress to compare the dose response curve of acetylcholine with the dose response curve of acetylcholine in the presence of a fixed dose of hypobranchial extract and on the frog rectus abdominus muscle. The dose response curve of acetylcholine will be compared with another curve, in which a selected dose of acetylcholine will be kept constant with increasing dose of the extract. This would confirm the presence or absence of the succinylcholine type of principle in the active fraction of this C. ramosus hypobranchial gland extract.

REFERENCES


