Insusceptibility to the Action of Cardiotonic Steroids of the 'Slow Muscle Fibre System' of the Toad

Since the work of VULPIAN[1] in the middle of the last century, it has become a well-established fact that the heart muscle of the toad is quite insensitive to the cardiotonic action of cardiac glycosides and their genins (to be called in the following 'cardiotonic steroids'). According to HERRMANN, PORTIUS and REPKE[2] and REPKE, EST and PORTIUS[3], this relative insensitiveness of the toad heart muscle towards cardiotonic steroids cannot be ascribed either to an inadequate absorption of these compounds by the heart muscle, or to a rapid metabolic breakdown and/or a rapid excretion, and may be explained on the basis of an insusceptibility of the receptor per se of the effector organ.

In contrast to these findings in the heart, the active transport of sodium in the toad bladder, as reflected in the short-circuit current, (SCC) was found by several investigators[4-6] to be inhibited by the cardiotonic steroids.

We reported previously[7] that the cardiotonic steroids could induce a sustained contracture of the slow skeletal muscle of the frog, *Rana nigromaculata*, through their action upon the small motor nerve. Under the influence of these compounds a certain change occurs within the nerve in close proximity to the nerve-muscle junction and, as a result, acetylcholine is liberated from its endings, which, in turn, produces a contracture of the muscle. In view of the above cited conflicting reports concerning the insusceptibility of the toad to the action of the cardiotonic steroids, it seemed to us of interest to know whether the cardiotonic steroids can induce a contracture in the slow skeletal muscle of the toad.

With a ventricular strip preparation of the frog, OTSUKA and NONOMURA[8] found a potentiation of the potassium contracture (K-contracture) by cardiotonic steroids. Since the relation between the membrane potential and the external potassium concentration was not appreciably affected by these compounds, they concluded that cardiotonic steroids altered the relation between the depolarization and contractile tension so that a smaller depolarization is required for the development of a given tension. Similar potentiation of K-contracture of the slow skeletal muscle fibres by cardiotonic steroids was observed in this laboratory using the rectus abdominis muscle of the frog[9]. In order to know whether the slow skeletal muscle fibres of the toad are sensitive to cardiotonic steroids or not, this phenomenon was made use of.

Experiments were performed at room temperature (15–25 °C) on the rectus abdominis muscle of the toad, *Bufo vulgaris*, obtained from May through October. A strip of the muscle, 15–25 mm long and about 5 mm wide, consisting of 1 or 2 segments was dissected out and suspended in an organ bath containing 10 ml of modified Ringer's solution aerated with 95% O_2 + 5% CO_2. The composition of the Ringer's solution was: NaCl 110 m*M*, KCl 2 m*M*, $CaCl_2$ 1.2 m*M*, $NaHCO_3$ 12 m*M*, glucose 5.5 m*M*. For comparison the rectus abdominis muscle of the frog, *Rana nigromaculata*, or the bullfrog, *Rana catesbiana*, was also used. Using a strain-gauge transducer, a carrier amplifier and a DC amplifier, the contraction of these preparations was recorded isometrically on an ink-writing oscillograph. In some experiments, the contraction of the preparation was recorded with a tension lever on a smoked drum.

Stock solutions of ouabain (g-strophanthin, Merck) was prepared dissolving 10 mg of this substance in 1 ml of redistilled water. Digitoxigenin was dissolved in 70% ethanol to make 1 mg/ml solution. Just before use these stock solutions were diluted with redistilled water to a desired concentration and a small volume of which (usually 0.1–0.3 ml) was added to the organ bath. The doses of acetylcholine chloride (ACh) used refer to the salt.

1. *Effect of cardiotonic steroids on the small motor nerve.* As illustrated in Figure 1, ouabain, in a dose of 3×10^{-6} mg/ml or more, could produce a definite con-

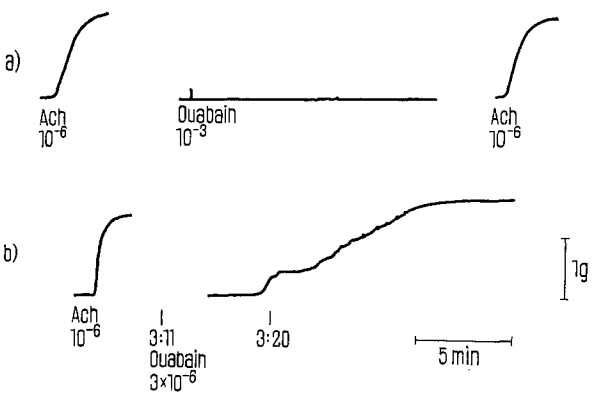

Fig. 1. Effect of cardiotonic steroids on the 'slow skeletal muscle fibre system' of the toad and the frog. a) Lack of the contracture-inducing action of ouabain (10^{-3} g/ml) in rectus abdominis muscle of the toad, *Bufo vulgaris*. b) Ouabain-contracture of rectus abdominis muscle of the frog, *Rana nigromaculata*.

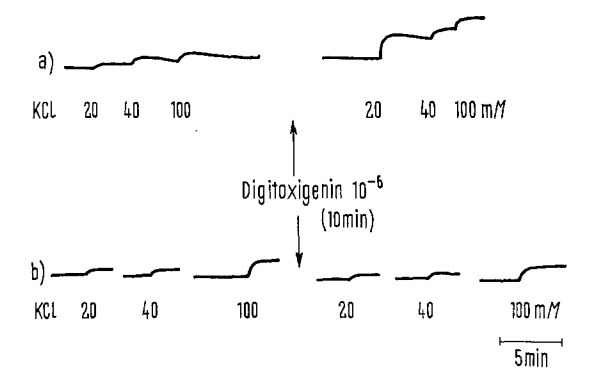

Fig. 2. Potassium contracture of rectus abdominis muscle and the effect of cardiotonic steroids on it. a) Potentiation of the contracture observed in the frog after administration of digitoxigenin (10^{-6} g/ml). To prevent the development of contracture by digitoxigenin itself, the muscle was treated previously with 10^{-8} g/ml of tetrodotoxin for 20 min. b) Lack of the potentiation by digitoxigenin in the toad.

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tracture of the slow skeletal muscle fibres of the frog, in agreement with the previous report from our laboratory[7]. In contrast, even after 10^{-3} g/ml of ouabain, no contractile response could be induced in the slow skeletal muscle fibres of the toad, although the muscle responded to 10^{-6} g/ml of ACh with a definite contracture, just as the slow skeletal muscle fibres of the frog did, indicating that the slow muscle fibres of the toad are not insensitive to acetylcholine. It may, therefore, be concluded that the observed lack of response of rectus abdominis muscle of the toad to ouabain resulted, not from the insusceptibility of the muscle to acetylcholine, but from the insusceptibility of the small motor nerve towards cardiotonic steroids.

2. *Potassium contracture of the slow skeletal muscle fibres.* Potassium contracture (K-contracture) of the slow skeletal muscle fibres was induced in the rectus abdominis muscle by replacing an equivalent amount of NaCl by KCl. It may be seen from Figure 2 that 10^{-6} g/ml of digitoxigenin was without effect on the K-contracture of the slow skeletal muscle of the toad, while there was a clear-cut potentiation of the K-contracture of the slow skeletal muscle of the frog.

Zusammenfassung. Untersuchungen der Wirkung von g-Strophanthin auf das Tonusfasersystem des Krötenherzens ergab selbst bei einer Konzentration von 10^{-3} keine kontraktile Reaktion. Auch die Kalium-Kontraktur der Tonusfasern wurde durch Verabreichung von Digitalis-Verbindungen nicht potentiert.

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Molecular Orbital Studies on the Conformation of γ-Aminobutyric Acid and Muscimol[1]

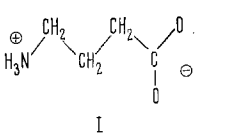
At the present time, a substantial amount of evidence is available which implicates γ-aminobutyric acid (GABA) (I) as a central inhibitory transmitter[2-6]. Evidence has also been presented which indicates that glycine may act as an inhibitory transmitter in the cat spinal cord[7-8] and on neurones in the caudate nucleus[9]. It was recently shown that GABA is considerably more active than glycine as an inhibitor of cortical neurones[10]. Other evidence suggests that glycine is probably not an inhibitory transmitter in the cortex of several species[11].

The role of neutral or neutrally charged endogenous amino acids, including GABA, in abnormal nervous conditions has been long sought[12-14]. These conditions include seizures associated with dietary deficiencies[15], epilepsy[16], and barbiturate abstinence convulsions[17].

It is conceivable that the design of substances with GABA-like activity capable of passing the blood-barrier may provide an approach to the supplementation of GABA deficiencies in these pathological conditions. In this regard, it is of interest that a substance with GABA-like central activity has been recently reported[18]. This compound, muscimol (II), is an isoxazole betaine found in the mushrooms of the genus *Aminata*[19].

The suggestion was made that, with the observation of the GABA-like action of muscimol, the examination of similar molecules with this type of conformational restriction might be rewarding in understanding the interaction of GABA and its receptor[18]. If GABA and muscimol act at the same receptor, and if their preferred conformations are pertinent to this interaction, then both GABA and muscimol should be able to readily exist in conformations in which comparably charged atoms or groups are presented to a receptor in a similar pattern[20].

In the present study, we have undertaken predictions of the preferred conformation of GABA and muscimol using molecular orbital theory. This same approach has been used in this laboratory to predict the conformational preference of numerous physiological mediating agents[20].



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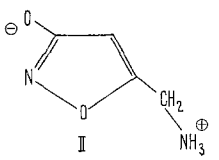

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