SPONTANEOUS ELECTRICAL ACTIVITY OF THE BULLFROG SPINAL CORD AND SUSCEPTIBILITY TO SOME CENTRALLY ACTING DRUGS AND AMINO ACIDS

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During a study of drug effects on the isolated perfused spinal cord of the bullfrog, spontaneous discharges were observed from the ventral roots (VII-XIth). It has been reported in frogs (1) and cats (1-3) that spontaneous electrical activity is led off with electrodes placed on or inserted into the spinal cord. Katz and Miledi (4) reported that spontaneous discharges were rare not only in the Xth segment of the frog spinal cord, where rhythmic bursts of efferent impulses were observed in several small motor axons. Spontaneous discharges from the ventral roots obtained in the present study appeared different in origin and nature from those reported by Katz et al. (4). In this paper characteristics of the discharges will be described with reference to the drug susceptibility.

The technique was essentially the same as described by Matsuura et al. (5, 6), who obtained reflex discharges as well as root potentials by stimulation of a dorsal root. The spinal cord with ventral and dorsal roots was carefully isolated and arranged in a chamber. As soon as possible, a glass cannula was inserted into the anterior spinal artery and then the spinal cord was perfused with O₂-aerated amphibian Ringer’s solution (16°±2°C, pH 7.6±0.2). The VIIth-XIth roots were mounted on the electrodes and served for stimulation or recording. Spontaneous discharges were amplified with an amplifier (Nihonkohden AVB-2) and displayed on an oscilloscope (VC-7). Discharges were then transformed into square waves and fed into an integrator, the output of which was recorded using a DC-recorder (Toa Electronics EPR-2TB). Reflex discharges were obtained from the ventral roots by stimulation of the dorsal roots and intracellular recording of the membrane potential of a motoneurone was made using KCl-filled glass electrodes.

Spontaneous discharges occurred within 1 hr after the beginning of perfusion of the spinal cord with Ringer’s solution and soon developed into irregularly repeated bursts with high frequency of a maximum of 700 Hz (Fig. 1 A). Bursts recorded from each ventral root almost synchronized with those recorded from others (Fig. 1 B) and lasted for more than 4 hr. The resting potential of the spinal motoneurone was approx. -60 mV. Spontaneous intracellular depolarizing potentials appeared in accordance with the extracellular ventral root discharges, and occasionally initiated action potentials (Fig. 1 C).

While decreasing the temperature of the preparation, the rate of discharge, expressed
FIG. 1. Spontaneous discharges recorded from ventral roots of the isolated perfused spinal cord of the bullfrog. 
(A) Rate of spontaneous discharge from ventral root. Vertical scale: Records of frequency (Hz) using integrator and DC-recorder. (B) Synchronous firing in roots. I VR10: Ipsilateral Xth ventral root, C VR10: Contralateral Xth ventral root, I DR9: Ipsilateral IXth dorsal root. (C) Simultaneous recording of the membrane potential of a motoneurone (upper tracings) and spontaneous discharges from the ventral root (lower tracings).

as a percentage of the value at 16°C, was reduced to 70% and 30% at 10°C and 5°C respectively. Even at low temperatures of 3°C–5°C, discharges did not disappear completely. Enhancement by cooling of electrical activity of the spinal cord in cats (7) was not observed in this preparation. When the temperature was increased up to 30°C, the rate of discharge gradually increased to 200%.

When normal Ringer's solution was replaced for a solution of lower pH, the rate of discharge tended to decrease. Close to a pH of 5.4, it was reduced to approx. 30% of that obtained at pH 7.6. When the pH was increased up to 9.0, a slight increase in the rate of discharge was observed. With further increase in alkalinity, there was a gradual increase in the rate followed by a sudden disappearance.

As shown in Fig. 2a, the addition of strychnine nitrate in a concentration of $1 \times 10^{-6}$ M to the perfusing medium resulted in a marked increase of the rate of discharge; each concentration of $1 \times 10^{-7}$ M or $1 \times 10^{-6}$ M also increased the rate. Even after the solution had been exchanged for drug-free Ringer's solution, some effects were still observed. The polysynaptic component of reflex discharges was enhanced with the application of strychnine nitrate ($1 \times 10^{-5}$ M), whereas the first spike was apt to be decreased by the same concentra-
Fig. 2. Effect of drugs on spontaneous discharges and reflex discharges recorded from the ventral root of the bullfrog.

Upper records (a, c and e) show the rate of spontaneous discharge from ventral root. Vertical scales: Records of frequency (Hz) using integrator and DC-recorder. Horizontal bar: Time for drug perfusion (20 min). Lower records (b, d and f) are five traces superimposed at a stimulation rate of 0.1 Hz. W60, W20 or W30 indicates time (min) after exchanging drug solution for drug-free Ringer's solution.

(a) and (b): Strychnine nitrate (1 x 10^{-5} M) (c) and (d): GABA (1 x 10^{-3} M) (e) and (f): Glutamic acid (1 x 10^{-3} M).

Almost complete inhibition of spontaneous discharges was obtained in a concentration of γ-aminobutyric acid (GABA) (1 x 10^{-3} M) (Fig. 2 c). Complete recovery from the effect of GABA was observed within 3 min after the solution had been exchanged for drug-free Ringer's solution. GABA (1 x 10^{-4} M) reduced the rate of discharge; 1 x 10^{-5} M exerted no or little effect. Depression of both the first spike and polysynaptic component of reflex discharges was at most 30% in a concentration of GABA (1 x 10^{-3} M) (Fig. 2 d).

In contrast, glutamic acid (1 x 10^{-3} M) caused a remarkable increase of the rate of discharge within 12 min. Following initial increase of the discharge, however, the excitatory effect of the amino acid was gradually reduced, although continuous firing still remained (Fig. 2 e). The effect disappeared after the solution had been exchanged for drug-free
Ringer's solution. Glutamic acid \((1 \times 10^{-3} M)\) enhanced to some degree the first spike and polysynaptic component of reflex discharges (Fig. 2 f).

In summary, spontaneous electrical activity was detected from the ventral roots of the isolated perfused spinal cord of the bullfrog and found to be susceptible to strychnine, picrotoxin, GABA and glutamic acid.

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EFFECT OF PROSTAGLANDINS \(E_1\) AND \(F_{2\alpha}\) ON HEART RATE BY DIRECT INJECTION INTO THE CANINE SINUS NODE ARTERY

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A number of investigators have found that intravenous injection of prostaglandin \(E_1\) (PGE\(_1\)) or prostaglandin \(F_2\)(PGF\(_2\)) increased heart rate in humans (Bergström et al. (1)) and in dogs (Nakano and McCurdy (2); Emerson et al. (3)). Lavery et al. (4) also showed that the intra-vertebral arterial injection of PGE\(_1\) and PGF\(_2\) also increased heart rate in dogs. Nakano and McCurdy (2) showed that the tachycardia induced by intravenous injection of PGE\(_1\) was completely abolished by the administration of propranolol (1 mg/kg).

The present study was undertaken to examine whether or not PGE\(_1\) or PGF\(_2\) has a direct effect on the sinus node by injecting the prostaglandins into an isolated sinus node artery.

Dogs weighing between 15 and 20 kg were anesthetized with sodium pentobarbital (30 mg/kg). After a mid-line neck incision, a T-tube was inserted into the trachea and the right hemithorax was opened under artificial respiration. The sinus node artery was isolated and perfused at a constant pressure (100 mm Hg) by means of a Sigmamotor pump (Model TMS) using the method described previously (Hashimoto et al. (5)). Systemic arterial pressure and heart rate were measured continuously by Statham pressure transducer Model P32A and electrocardiograph, while recording was done using a Grass Polygraph