Effect of Nicotine on the Transmembrane Potential and Contractility of Isolated Rat Atria

It has been reported that nicotine increased the uptake and release of radiocalcium from frog sartorius[1,2] and toad ventricular muscle[3]. This would suggest that nicotine changed the ionic permeability of cellular membranes, which would result in recorded positive inotropic action. However, the effects of nicotine on the transmembrane potentials of cardiac membranes are unknown. The primary purpose of the present investigation was to establish correlations between electrical and mechanical changes in cardiac cells under the influence of nicotine.

Isolated rat atria were electrically excited at a rate of 200 pulses/min in Krebs-Ringer bicarbonate medium. Membrane potentials and mechanical activity were determined simultaneously using microelectrodes and a strain gauge as previously described[4,5].

The tissue medium was aerated with a mixture of 95% O_2 and 5% CO_2, maintained at 30 °C and pH 7.4. A constant diastolic tension of 750 mg was maintained on the tissue by means of a micrometer head. Microelectrode penetrations were made into the endocardial wall of prepared left atrium in regions approximately equidistant from the stimulating electrodes.

Each experiment consisted of 4 phases: (1) a 60 min equilibration period, (2) a 30 min period during which control records were taken, (3) a 30 min period at the beginning of which the drug was administered and records were taken at 3–5 min intervals, and (4) a wash-out period during which the drug was replaced with normal medium and the reversibility of the drug-induced responses were recorded. Nicotine alkaloid (Eastman Kodak) was diluted with Krebs-Ringer bicarbonate medium to provide the following concentrations: 5×10^{-5}, 1×10^{-4}, and $5 \times 10^{-4} M$.

The responses of rat atria to the test concentrations of nicotine are summarized in the Table. Nicotine, $5 \times 10^{-5} M$, was without any statistical significance on any of the measured characteristics, whereas the higher concentrations employed significant changed atrial characteristics. It can be seen that $5 \times 10^{-4} M$ nicotine increased the action potential duration and its inscribed area. These effects usually occurred within 5 min after drug administration. It was found that there was a parallel change in the action potential duration and area when compared with the developed tension. Subsequent exchange of the drug with normal medium resulted in a complete restoration of all the measured characteristics to initial control values.

The effects of nicotine on the mechanical activities of skeletal muscle[2,6,7] and cardiac muscle[8–11] have been well documented. However, there are no published reports on the actions of nicotine on the transmembrane potentials of cardiac muscle. In the present study $1 \times 10^{-4} M$ nicotine changed the membrane potentials without altering rat atrial contractility, whereas $5 \times 10^{-4} M$ nicotine was able to induce a positive inotropic action without a time correlated action on the resting potential or action potential. These results would indicate that the contractile effects induced by nicotine were not related to changes in the resting and action potential magnitudes. It has been reported that nicotine induced contraction in frog muscle was not inhibited by soaking the muscles in Ringer solution in which *Tris* or sucrose was substituted for Na^+ ions[2]. This would suggest that while nicotine may influence membrane activity, its action may also be directly on the contractile system. The apparent correlation found in the studies reported here was between the action potential duration and area with the contractile magnitude. It was found that the action potential depolarization rate remained fairly unchanged. It was suggested previously that the rate of repolarization was of major importance in the determination of the contractile response of rat atria[5]. While other reported works have been concerned mainly with attempting to correlate

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Effect of nicotine on isolated rat atria

Parameters	Control	Concentration of nicotine				
		$5 \times 10^{-5} M$	$10^{-4} M$	$5 \times 10^{-4} M$		
		0–30 min[a]	0–30 min	0–10 min	10–20 min	20–30 min
Resting potential, mV	62.5 ± 1.0[b]	63.0 ± 0.8	65 ± 0.8[c]	63.5 ± 1.0	66.7 ± 1.3[c]	67 ± 1.4[c]
Action potential, mV	71.1 ± 1.3	71.5 ± 1.0	76.4 ± 1.8[c]	73.5 ± 0.8	80.3 ± 1[c]	78 ± 1.2[c]
Action potential duration, msec	43 ± 1.3	43.7 ± 2.0	44.5 ± 1	47.8 ± 1.8[c]	51 ± 1.5[c]	50.4 ± 2[c]
Action potential area, mV-sec	1.8 ± 0.05	1.86 ± 0.01	1.9 ± 0.07	1.98 ± 0.02[c]	2.0 ± 0.01[c]	21 ± 0.02[c]
Depolarization rate, V/sec	39.1 ± 1.2	40 ± 0.8	39.1 ± 0.2	38 ± 0.8	39 ± 0.6	40 ± 0.4
Conduction time, msec	10.7 ± 0.3	11 ± 0.2	10.8 ± 0.16	10.2 ± 0.4	10.5 ± 0.5	11 ± 0.3
Latent period, msec	20.7 ± 0.6	19.7 ± 0.4	20.3 ± 0.45	20 ± 0.4	19.8 ± 0.6	20 ± 0.5
Developed tension, mg	347.6 ± 7	350 ± 5	352.1 ± 4.2	389 ± 6[c]	385 ± 7[c]	378 ± 7[c]
Developed tension duration, msec	72.2 ± 1.2	72.6 ± 1.0	71.6 ± 1.0	72 ± 2	72 ± 1.6	73 ± 2.2
Developed tension rise time, msec	42 ± 0.1	42.8 ± 0.5	42.5 ± 0.2	42 ± 1.2	43 ± 0.9	42 ± 1.2
No. of penetration	321	108	111	38	40	37
No. of atria	12	4	4	4	4	4

[a] Reading interval following drug application. [b] Mean ± S.E.M. [c] Values were significantly different from control.

changes in resting or action potential magnitudes with developed tension[12–14].

The precise mechanism of drug actions cannot be determined through the use of an electrical approach; but specific inferences may be drawn. These would be the relationships between flux studies and configurations of the action potentials. In the present study the observed facts suggest that nicotine may alter contractility by changes in the duration of the action potential, which implies alterations in potassium fluxes.

Zusammenfassung. Die Wirkungen von Nikotin auf die Kontraktilität und die Membranpotentiale des Rattenatriums wurden untersucht. Nikotin ($5 \times 10^{-4} M$) erhöhte gleichzeitig die entwickelte Spannung sowie die Dauer und Fläche des Aktionspotentials. Ein möglicher Zusammenhang dieser Änderungen mit der erhöhten Kontraktilität wird diskutiert.

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Influence of Prenatal X-Radiation on Brain Lipid and Cerebroside Content in Developing Rats

Prenatal X-radiation markedly alters the functional development of the central nervous system[1]. Histological[2] and biochemical[3] studies in rats have shown that exposure to X-radiation at 14 days of gestation increases the proliferation of glial cells in the developing brain of the offspring. Since glial cells are implicated in the myelination of the CNS[4], alterations in glial cell number may affect the process of myelination, which, in turn, would influence CNS development.

The present study, therefore, was designed to investigate in the rat the effects of prenatal X-radiation on brain lipids and cerebrosides, which are the components of the myelin sheath.

Eight pregnant Long-Evans rats were exposed to a single dose of 100 R whole-body X-radiation at 14 days of gestation. The pregnant animals were placed in individual open-ended cylindrical lucite containers and were rotated on a movable platform 80 cm from the X-ray source, a Picker 180 kV, 15 mA X-ray therapy unit using 0.5 mm Cu and 1.0 mm Al filters. The dose rate, 19 R/min, was measured in air by means of a Victoreen condenser R-meter which was placed inside the lucite container at the level of the animal's body. In order to eliminate any changes due to maternal stress other than X-radiation, 8 pregnant animals were sham-irradiated for the same length of time and served as controls. At delivery, the litters of X-irradiated rats were similar in number and size to those of controls.

The animals were sacrificed by decapitation at time periods of 9, 12 and 16 days after birth. The whole brain was immediately removed, dissected free of grossly visible blood vessels, blotted free of moisture and weighed. The cerebral cortex and diencephalon midbrain were then dissected and weighed. Water content in samples of these structures was determined by drying at 104 °C under vacuum for 4 days.

Lipids were extracted from samples of cerebral cortex and diencephalon midbrain with 2:1 chloroform/methanol, according to the method of FOLCH et al.[5]. Lipids excluding gangliosides were determined gravimetrically. Values for lipids were expressed as mg/100 mg of wet tissue. The method described in ROUSER et al.[6] for the fractionation of brain lipids on Florisil columns was adapted for small amounts of lipids. Two components of the cerebroside molecule, galactose and sphingosine, were analysed. For the isolation of galactose and sphingosine the column eluants which contained the cerebroside were subjected to acid hydrolysis[7,8]. Sphingosine was determined by the method of LAUTER[9] and galactose was determined by the orcinol-sulphuric acid reaction[10]. The galactose content of the sample was read directly from a standard curve and was multiplied by 4.66 to give the actual value of the cerebrosides. A mean molecular weight of 846 was used for the calculation of cerebrosides. Values for cerebrosides were expressed as mg/100 mg of lipid.

To determine whether the parameters measured in control and irradiated rats differ significantly in their means, the *t*-test for non-paired data was applied[11].

Body weights of prenatally X-irradiated rats did not differ from those of control rats at any of the age periods studied (Table I), whereas brain weights of irradiated rats were markedly lower compared to controls at all age periods (Table I). No differences were observed in the

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