Propranolol Blocks the Extrasynaptic Nicotinic Receptors on the Intramural Cardiac Ganglion Cells

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Nicotine has long been characterized as having two phases of action on the heart, initially negative and subsequently positive chronotropic and inotropic effects. The nicotine-induced inhibition has been generally assumed to be due to activation of the nicotinic receptors on the cardiac ganglion cells that fires the axons, liberates acetylcholine (ACh) and eventually inhibits atrial contractions (Giotti, 1954;

Leaders and Long, 1962; Chiang and Leaders, 1965; Khan et al. 1965; Long and Gross, 1966; Chiang and Leaders, 1968). Odawara and Nishi (1980) have shown, however, that the nicotine-induced inhibition of the isolated guinea-pig atrium can occur after blockade of the nerve conduction by tetrodotoxin. They reported that the axon terminals of cardiac ganglion cells are endowed with nicotinic recep-

Fig. 1. Alteration by propranolol of the nicotinic responses of intramural ganglion cells isolated from guinea-pig atrium. *a-c*: Fast EPSPs of a neuron elicited at membrane potential of -80 mV by single focal stimuli. *d-f*: Responses (fast ACh potentials) of another neuron at a resting membrane potential of -52 mV induced by electrophoretic application of ACh by pulses of $0.1 \mu\text{A}$ with a duration of 30 msec. Records were taken intracellularly before (*a* and *d*) and 5 min after (*b* and *e*) addition of propranolol ($5 \mu\text{M}$) to the perfusing solution and 15 min after (*c* and *f*) washing out propranolol.

tors, and activation of these receptors by nicotine causes liberation of the endogenous ACh which then inhibits atrial contractions. Imawatari (1982) found that propranolol reversibly blocks the nicotine-induced inhibition of guinea-pig atrium and suggested that propranolol suppresses the nicotinic receptors at the axon terminals.

We have recently investigated the effect of propranolol on the nicotinic responses of the intramural cardiac ganglion cells and reached a conclusion that propranolol selectively inhibits the extrasynaptic nicotinic receptors.

The intramural ganglia were isolated from the guinea-pig atrium and super-

fused *in vitro* at $36 \pm 1^\circ\text{C}$. Single ganglia were fixed to the bottom of the perfusing chamber covered with a thin layer of Sylgard (Dow Corning). The fixed ganglion has intact neural connections with the remainder of the intramural plexus. Neurons were directly observed during intracellular recording using Nomarski optics ($500\times$). The full methods of fixation, perfusion, intracellular recording, focal stimulation of the ganglion, and ACh electrophoresis were similar to that described by Nishi and North (1973).

As illustrated in Fig. 1, propranolol ($5\text{ }\mu\text{M}$) markedly depressed the fast ACh potential induced by ionophoresis, while it only slightly depressed the fast EPSP

Fig. 2. Dose-response relationship for the fast ACh potential in the presence and absence of propranolol. Reciprocals of the fast ACh potential (ordinate) and electrophoretic current intensity (abscissa) are plotted. Values were obtained from the same neuron before (open circles) and 5-8 min after (close circles) addition of propranolol ($2\text{ }\mu\text{M}$). The resting membrane potential was -52 mV . The amplitude of the fast ACh potential was corrected by Martin's method (1955).

elicited by focal stimulation. When the fast EPSP was elicited at a hyperpolarized level (e. g., -80 mV) of membrane potential, a hump was observed on the falling phase of the EPSP near its peak. Thus, the falling phase had an initial fast decay phase (time constant, 5 to 8 msec), a hump and a slow decay phase (time constant, approximately 15 msec). Propranolol (5-10 μ M) eliminated the hump and the slow decay phase, leaving only the fast phase. It might be anticipated that the hump and the slow decay phase of the fast EPSP are produced by activation of the extrasynaptic receptors by the liberated ACh, and that the short-lasting EPSP remaining in the presence of propranolol is generated by activation of the subsynaptic receptors.

The dose-response relationship for the fast ACh potential during propranolol (2 μ M) application appeared to be that of non-competitive type (Fig. 2). This suggests that propranolol acts on the allosteric site or the ionic channels associated with the extrasynaptic receptors.

Thus it is most likely that there exist two kinds of nicotinic receptor on cardiac ganglion cells; one with a fast kinetics and hardly sensitive to the blocking action of propranolol and the other with a slow kinetics and highly sensitive to propranolol. It would be reasonable to assume that the nicotinic receptors at the intramural axon terminals are similar, if not identical, to the extrasynaptic nicotinic receptors on the ganglion cell somata. Based on these considerations, abolition by propranolol of the nicotine-induced atrial inhibition would be primarily due to suppression of the nicotine-induced depolarization at the post-ganglionic axon terminals.

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