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FREQUENCY-DEPENDENT DEPRESSION OF GANGLIONIC TRANSMISSION BY PROPRANOLOL AND DILTIAZEM IN THE SUPERIOR CERVICAL GANGLION OF THE GUINEA-PIG

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1 Effects of propranolol and diltiazem on ganglionic transmission in the superior cervical ganglion of the guinea-pig were investigated with intracellular recording techniques.

2 Propranolol and diltiazem (5×10^{-6}–10^{-5} M) induced a transmission failure in the ganglion upon preganglionic nerve stimulation at high frequency (25–30 Hz) without affecting action potentials induced by direct stimulation of the soma membrane, or potentials induced by iontophoretically applied acetylecholine.

3 The results suggest that propranolol and diltiazem may act on preganglionic nerve terminals to inhibit Ca^{2+} influx in a frequency-dependent manner. These agents may depress excess sympathetic activity without much affecting normal ganglionic transmission.

Introduction

It is known that calcium currents (I_{Ca}) play an important role in a variety of functions as diverse as excitation-contraction coupling in cardiac or smooth muscle and excitation-secretion coupling at presynaptic nerve endings and exocrine gland (Fatt & Katz, 1953; Katz & Miledi, 1967; Rubin, 1974; Hagiwara, 1975; Meech, 1976; Putney, 1978). Consequently, agents that impede the translocation of Ca^{2+} from the external medium to the cell interior would be expected to alter the function of such organs and tissues. In fact, organic Ca^{2+}-antagonists have been shown to modify the functions of cardiac and smooth muscle (Kohlhardt, Bauer, Kraus & Fleckenstein, 1972; Granefield, Aronson & Witt, 1974; Bayer, Kaufmann & Mannhold, 1975; Golenhofen, 1976; Fleckenstein, 1977; Taira, 1979; Payet, Schanne, Ruiz-Ceretti, 1980; Tajima, Kanda, Kitamura, Ito & Kuriyama, 1980). Recently, we have shown that propranolol inhibits the voltage-dependent I_{Ca} in *Helix* neurones in a manner similar to that of organic Ca^{2+}-antagonists (Akaike, Nishi & Oyama, 1981; Akaike, Brown, Nishi & Tsuda, 1981; Akaike, Ito, Nishi & Oyama, 1982). This prompted us to examine the effects of propranolol and a Ca^{2+}-antagonist, diltiazem, on synaptic transmission in the mammalian ganglion, since the release of transmitter from the nerve terminals of sympathetic ganglia is also considered a Ca^{2+}-dependent process (Katz, Miledi, 1973; Bennett, 1973).

Methods

The superior cervical ganglion together with its preganglionic nerve trunk was removed from adult guinea-pigs of either sex, anaesthetized with sodium pentobarbitone, and transferred to a superfusion chamber (total volume about 1 ml). The preparation was superfused with normal mammalian saline of the following composition (mM): NaCl 120, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25 and glucose, 5.5; the solution was equilibrated with a gas mixture of 95% O_2 and 5% CO_2 and maintained at 34°C–36°C. The preganglionic nerve trunk (about 1 cm) was lifted into a layer of mineral oil (covering the flowing saline) onto a pair of platinum electrodes for orthodromic stimulation. Rectangular pulses of 0.1 ms duration and of submaximal strength were applied.

Intracellular recordings were made, using conventional microelectrode techniques. An electrometer (WPI M701) permitted current injection into the cells through the recording electrode. Intracellularly recorded potentials and current pulses were displayed on independent channels of an oscilloscope (Tektronix Type 502A) and simultaneously fed to independent channels of an FM tape recorder (TEAC R410) for further analysis and photography. The technique for iontophoretic application of acetylcholine (ACh) was that described by Christ & Nishi (1971).

Drugs employed in the present experiments were:

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propranolol hydrochloride (ICI), diltiazem [3-acetoxy-2, 3-dihydro-5-2-(dimethylamino)ethyl]-2(4-methoxy-phenyl)-l, 5-benzothiazepin-4(5H) HCl (Tanabe Pharm. Co., Japan) and acetylcholine hydrochloride (Merck). They were dissolved in test solution just before use.

Results

Resting membrane potentials (MPs) of ganglionic cells were considered adequate only when the cells gave action potentials (APs) larger than 55 mV. Upon electrical stimulation of the preganglionic nerves, multiple potentials consisting of e.p.s.ps immediately followed by APs were recorded. When a depolarizing current pulse was injected through the recording electrode, one or a train of APs was produced by stimulus voltage beyond a threshold level. The configuration and time course of the e.p.s.p. and AP were similar to those previously reported (Eccles, 1955; Erulkar & Woodward, 1968; Christ & Nishi, 1971).

Effects of propranolol and diltiazem were tested in 12 cells (MP −50 to −70 mV; AP −55 to −80 mV) held for periods of 30–120 min (5 cells for propranolol and 7 cells for diltiazem). Propranolol and diltiazem at a concentration of 10^{-6} M did not alter MP, nor the threshold for preganglionic and direct stimulation. When higher concentrations of these agents (5×10^{-6}–10^{-5} M) were applied, the amplitude of the e.p.s.p. elicited by a single preganglionic stimulus was slightly decreased, but APs were still elicited. At the end of a 30 min period of drug application, there was no appreciable cell depolarization, nor change in the rate of rise and amplitude of APs elicited either by indirect or direct stimulation. Diltiazem (10^{-5} M) exerted similar effects on the e.p.s.p. However, when preganglionic nerves were stimulated repetitively (30–50 Hz), these agents induced

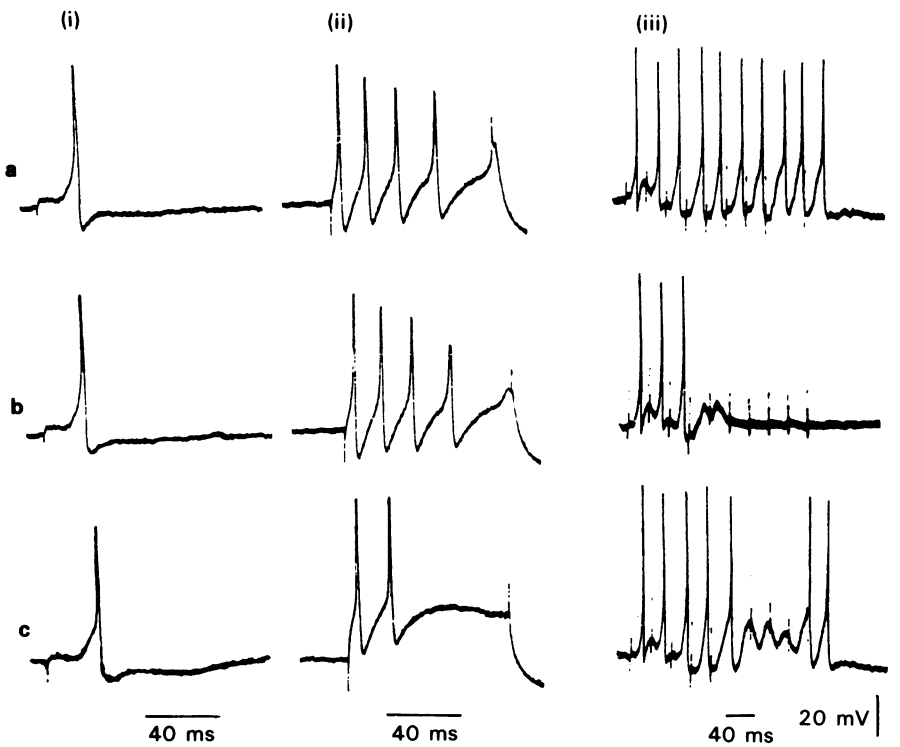

Figure 1 Effects of propranolol and diltiazem on orthodromic and directly-evoked action potentials. (a) Control: (i) action potential evoked by a single pulse applied to preganglionic nerves; (b) action potentials evoked by passing current (0.5 nA) across the cell membrane; (iii) action potentials evoked by a train of stimuli applied to the preganglionic nerves at 33 Hz. (b) Propranolol 10^{-5} M: (i) 30 min after beginning superfusion with propranolol; (ii) immediately after (b)(i). (iii) 1 min after (b)(i). Records (a) and (b) were taken from same cell. After a 30 min period of washing, the preparation showed complete recovery. (c) Diltiazem 10^{-5} M: (i) 10 min after beginning superfusion with diltiazem; (ii) immediately after (c)(i); (iii) 1 min after (c)(iii). Records during control period omitted, since the responses to orthodromic and direct stimulation were almost the same as (a)(i) obtained from a different cell.

marked depression of ganglionic transmission. During the control period, repetitive orthodromic stimulation at various intervals of 20–100 ms induced a train of APs in a one-to-one manner. In the presence of propranolol (10^{-5} M), the postsynaptic membrane responded only to the initial phase of stimulation (30–50 Hz), eliciting 3–4 APs. The amplitude of successive e.p.s.ps was markedly reduced (Figure 1 (b)(iii)). Diltiazem (10^{-5} M) also depressed ganglionic transmission at high frequency stimulation (30–50 Hz). The amplitude of e.p.s.ps induced by orthodromic stimulation showed considerable variations, and some of the e.p.s.ps were subthreshold, causing an occasional failure of initiation of APs in the postsynaptic membrane (Figure 1 (c)(iii)).

In four cells, ACh was applied by iontophoresis to the postsynaptic membrane. The ACh-potentials obtained in the presence of propranolol or diltiazem (10^{-5} M) were not significantly different from those obtained in the control without drugs.

Discussion

Propranolol and diltiazem at relatively low concentrations (5×10^{-6}–10^{-5} M) depressed ganglionic transmission, when the preganglionic nerves were stimulated at high frequency. The depressant effects of propranolol could have been due to block of facilitatory β-adrenoceptors. This seems unlikely since catecholamines decrease the amplitude of e.p.s.ps in sympathetic ganglia (Weir & McLennan, 1963; Christ & Nishi, 1971). Another possibility is that nerve impulses in the preganglionic nerve might be blocked by the local anaesthetic property of propranolol (Sasa, Avner & Albuquerque, 1973; Ishida, Sasa & Takaori, 1980). However, propranolol at the concentrations employed in the present experiments did not cause any appreciable changes in APs in the postsynaptic membrane. Therefore, it is most likely that the action of propranolol may be related to inhibition of Ca^{2+} influx at the nerve terminals as in the case of diltiazem and other Ca^{2+}-antagonists. In fact, diltiazem reduces the amplitude of excitatory junction potentials in the vas deferens, possibly acting on the nerve terminals and inhibiting Ca^{2+} influx (Tajima *et al.*, 1980). It is suggested that interference with Ca^{2+} influx may be especially critical when the terminal membrane is depolarized repetitively, resulting in a decrease in the amplitude of successive e.p.s.ps.

It is premature to relate the present results to the clinical application of these drugs, but we propose that propranolol and other organic Ca^{2+}-antagonists may suppress excess sympathetic activity without much affecting normal ganglionic transmission.

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