Br. J. Pharmac. (1981), **73**, 431–434

INHIBITORY EFFECTS OF PROPRANOLOL ON THE CALCIUM CURRENT OF *Helix* NEURONES

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1 Calcium current (I_{Ca}) and potassium current (I_K) in isolated nerve cell bodies of *Helix aspersa* were independently separated after suppression of Na^+ and K^+ currents, and Na^+ and Ca^{2+} currents, respectively. The suction pipette method was used. Under voltage clamp conditions, the effects of propranolol on both the I_{Ca} and I_K were examined.

2 Propranolol inhibits the I_{Ca} at relatively low concentrations (10^{-6}–10^{-5} M). The inhibitory action was dose-dependent.

3 The I_K was moderately suppressed by the drug at high concentrations (10^{-5}–5×10^{-4} M).

4 Results provide evidence for a new aspect of the pharmacological action of propranolol on the excitable cell membrane.

Introduction

Propranolol and certain other β-adrenoceptor blocking agents (β-blocker) have a substantial local anaesthetic activity on the cardiac cell (Gill & Vaughan Williams, 1964; Morales-Aguilerá & Vaughan Williams, 1965; Barrett & Cullum, 1968). Recently, Hashimoto, Satoh & Imai (1979) suggested a possible inhibitory action of propranolol on the Ca^{2+}-channel of the cardiac cell membrane. However, actions of propranolol and other β-blockers known to have local anaesthetic actions on each single ionic current (I_{Ca}, I_{Na} and I_K) across the excitable cell membrane have not yet been analysed in detail.

The transmembrane currents of molluscan neurones are carried by Na^+, Ca^{2+} and K^+ (Thompson, 1977). Lee, Akaike & Brown (1978) have shown that the three ionic currents in snail neurones can be separated by using a suction pipette technique which allows voltage clamp and internal perfusion. In the present work the suction pipette technique was used to investigate how propranolol affects I_{Ca} and I_K of the *Helix* neurone.

Methods

The experimental method was essentially similar to that previously described (Lee *et al.*, 1978). Briefly, the experiments were performed on neurones located in the oesophageal ganglion of *Helix aspersa*. The ganglion was removed and connective tissue was stripped off with fine forceps until clusters of neurones floated free in 'normal' snail Ringer having the following composition (mM): NaCl 85, KCl 5, $CaCl_2$ 10, $MgCl_2$ 15, Tris-HCl 5 and glucose 5.5; pH 7.5. A part of each individual neurone (50–100 μm in diameter) was then sucked into the 15–30 μm diameter tip of a suction pipette under a negative pressure of more than −300 mmHg and then the cell body was isolated from residual connective tissue and the axon (Lee, Akaike & Brown, 1980). Internal perfusion was preceded by disrupting part of the neuronal membrane aspired into the tip of the suction pipette. The ionic composition of the normal solution used for internal perfusion was K-aspartate 135 mM, buffered with Tris-base at pH 7.4. Ionic currents were monitored on a storage oscilloscope (Tektronix 5113) and simultaneously recorded with a photosensitive paper recorder system (Medelec, MS6). Holding potential (V_H) was usually −60 mV for I_{Ca} and −40 mV for I_K and currents produced by each of a pair of depolarizing and hyperpolarizing pulses of equal amplitude were added to give I_{Na}, I_{Ca} or I_K free of leakage and shunt currents and the linear component of the capacitative current, using a signal averager (Medelec, DAV62). The solutions for separating I_{Ca} had the following composition (mM): external solution, Tris-Cl 35, tetraethylammonium chloride 50, CsCl 5, $MgCl_2$ 15, $CaCl_2$ 10, 4-aminopyridine 5 and glucose 5.5, pH 7.5; internal solution, Cs-aspartate 135, tetraethylammonium hydroxide (TEA-OH) 10, buffered with EGTA-Ca 0.1 ($[Ca]_i < 10^{-8}$ M), pH 7.4. For separating I_K from I_{Ca} and I_{Na}, $CaCl_2$ and NaCl were replaced with $CoCl_2$ and Tris-Cl, respectively. A transient K^+-current (I_A) was inhibited by addition of 4-aminopyridine (5 mM) to the external solution and use of a holding potential of −40 mV. The internal solution for I_K was K-aspartate 135 mM,

0007-1188/81/060431-004 $01.00

buffered with Tris base at pH 7.4. Effects of (±)-propranolol (ICI) on ionic currents were examined by adding the drug to the bathing medium. However, the major inward current in the *Helix* neurone is I_{Ca} and most of the neurones have little I_{Na}. In the present experiments, therefore, effects of propranolol on both I_{Ca} and I_K were mainly examined in detail. All experiments were carried out at room temperatures of 20–23°C.

Results

Effects of propranolol on I_{Ca}

Figure 1a shows actual Ca^{2+}-current records obtained under voltage clamp from a neurone in Na^+, K^+-free, Tris, Cs^+ solutions. When I_{Na} and I_K were blocked by external and internal perfusion with the solutions described in Methods, a slowly rising inward current of I_{Ca} appeared at depolarizing voltage steps of 10–15 mV from the holding potential of −60 mV. This rose smoothly, reached its peak within 5–10 ms, and persisted over the subsequent 15–100 ms. At higher depolarizing voltages, a transient peak current appeared at 1–3 ms, which declined and merged with a slowly inactivating current (Akaike, Lee & Brown, 1978). After ensuring that there were no changes in the I_{Ca} current-voltage (I-V) relationship during the control period of 30 min, the preparation was superfused with the external solution containing propranolol at a flow rate of 5 ml/min in a 1 ml perfusion chamber. Five min after the start of the superfusion, I-V curves of I_{Ca} in the presence of propranolol at various concentrations were obtained (Figure 2a). Dose-response curves for the inhibitory effects of propranolol are shown in Figure 3. At a concentration lower than 10^{-7} M, propranolol exerted no effect on I_{Ca} but started to inhibit the I_{Ca} at a concentration of 10^{-6} M. At this concentration the inhibition reached a maximum within 5 min at which time the peak I_{Ca} was depressed by 10–15% of the control (12 ± 2%, mean ± s.e. mean, n=5). A further increase in the concentration of propranolol inhibited the I_{Ca} dose-dependently and did not shift the I-V relationship; the ratios of the inhibition of the drug at concentration of 10^{-5} M, 10^{-4} M and 5×10^{-4} M were 25 ± 3% (n=5), 72 ± 5% and 92 ± 7% (n=5), respectively, 5 min after the drug application. With propranolol 10^{-4} M, the I_{Ca} was inhibited progressively as the time of the application was prolonged and was markedly depressed within 15 min. At concentrations higher than 10^{-5} M, the inhibitory effect of propranolol persisted for 2–3 h, even after washing it out. However, when the preparation was exposed to propranolol (10^{-5} M) for less than 5–7 min, the I_{Ca} recovered to about 80% of the control value within 2 h. The drug inhibited the I_{Ca} without affecting the activation and inactivation processes of the I_{Ca} (Figure 1a).

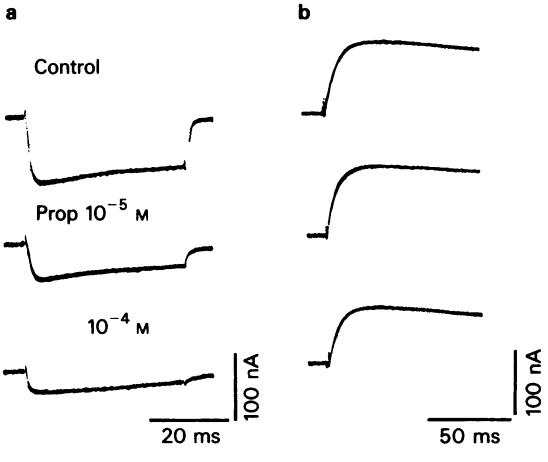

Figure 1 Effects of propranolol on the calcium (I_{Ca}) and potassium (I_K) currents in the *Helix* neurone. (a) Effects of propranolol on peak I_{Ca} elicited by voltage steps of +20 mV from the holding potential of −50 mV. (b) Effects of propranolol on peak I_K elicited by voltage steps of +80 mV from the holding potential of −40 mV. Sample records of I_{Ca} and I_K were obtained after subtraction of leakage, shunt and linear capacitative currents. From top to bottom; control, 5 min after application of propranolol (Prop) of 10^{-5} M and 10^{-4} M, respectively. (a) and (b) were obtained from different experiments.

Effects of propranolol on I_K

The preparations were superfused with solutions for separating I_K for 10–20 min and then voltage steps of 10 mV from the holding potential of −40 mV were applied. The outward current of I_K appeared at −20 to −10 mV and developed progressively. Peak of the I_K appeared at approx. 10–15 ms (Figure 1b). The I_K current-voltage (I-V) relationship was obtained during the control period of 30 min. Thereafter, the preparation was superfused with the external solution containing propranolol at various concentrations. Propranolol did not affect the I_K at a concentration lower than 10^{-6} M but started to inhibit the I_K at a concentration of 10^{-5} M; the ratio of depression was 6% of the control (n=6). At higher concentrations (10^{-4}–10^{-3} M), a substantial depression of the I_K was observed 5 min after the start of the drug-application (Figure 3). The inhibitory effect of the drug persisted for 2–3 h after washing the preparation. The depression of the I_K was pronounced at larger depolarizations (see Figure 2b).

Discussion

The present experiments have demonstrated that

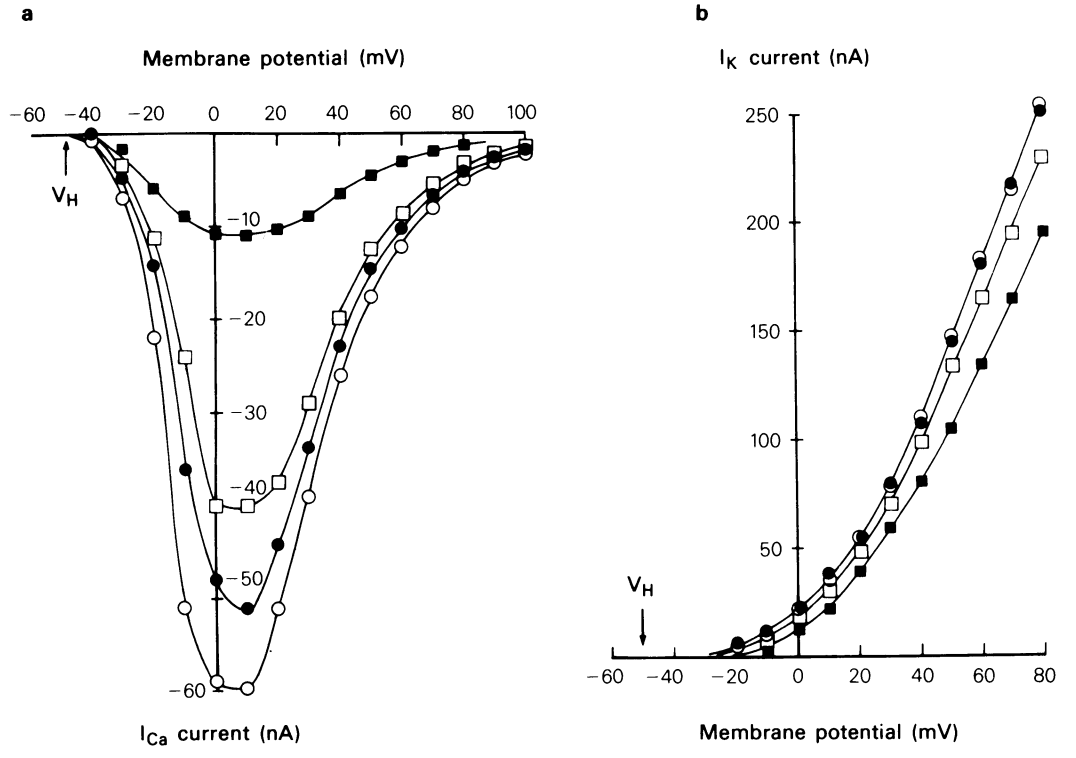

Figure 2 Effects of changes in concentrations of propranolol on current-voltage relationship of I_{Ca}. In (a) and (b): (○) control; (●) 5 min after application of propranolol 10^{-6} M; (□) 10^{-5} M; (■) 10^{-4} M. V_H = holding potential. (a) and (b) were obtained from different experiments.

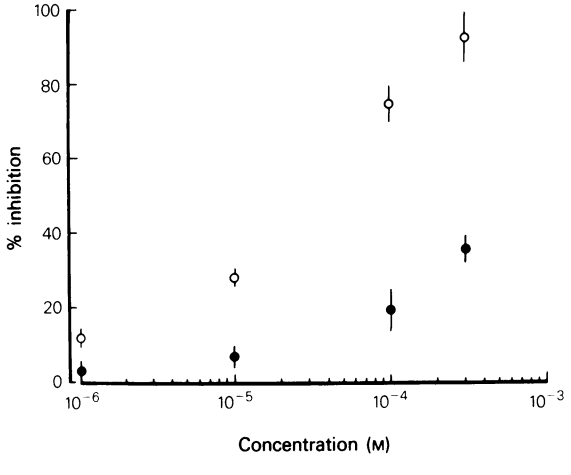

Figure 3 Dose-response curves for inhibitory ratios of I_{Ca} (○) and I_K (●) after application of propranolol. The % inhibition was calculated from peak current before and after drug application. Each point indicates the average value of 5–6 experiments and vertical bars show s.e. mean.

relatively low concentrations of propranolol (10^{-6}–10^{-5} M) inhibit the I_{Ca} but have little effect on the I_K in the *Helix* neurone. There is the possibility that the inhibitory effect of the drug on the I_{Ca} could be due to secondary effects resulting from an intrinsic β-adrenoceptor blocking action but only if it is assumed that under normal circumstances the I_{Ca} might be modified through the β-adrenoceptors by a transmitter substance continuously released from nerve endings impinging on the cell body. However, this is unlikely since in the present experiments each nerve cell body was completely separated from other surrounding neural elements and rendered free of its axon. Therefore, it is reasonable to conclude that the inhibitory action of propranolol on both I_{Ca} and I_K is due to a direct action on the soma membrane.

From previous studies, local anaesthetics are known to inhibit the early transient (I_{Na}) and late steady state (I_K) currents in the nerve membrane (Taylor, 1959; Shanes, Freygang, Grundfest & Amatniek, 1959; Blaustein & Goldman, 1966; Narahashi, Moore & Piston, 1969). Propranolol also exerts local anaesthetic actions on the lobster axon (Sasa, Avner & Albuquerque, 1973) and the crayfish

giant axon (Ishida, Sasa & Takaori, 1980); the drug inhibits the dv/dt and amplitude of the action potential initiated by I_{Na} in a dose-dependent manner. The present results provide evidence that propranolol possesses another pharmacological action, different from those of local anaesthetics on the excitable cell membrane. In this regard, the mode of action of propranolol on I_{Ca} was rather similar to that of organic Ca^{2+}-antagonists such as verapamil and diltiazem on *Helix* neurones, and the concentration of the drug required to inhibit I_{Ca} was also in the same range as that of the Ca^{2+}-antagonists (Akaike, Lee & Brown, 1979). Thus, the potency of propranolol in inhibiting I_{Ca} is comparable to that of the Ca^{2+}-antagonists.

Fleckenstein (1964) has shown that the depressant effect of a certain type of β-blocker on the contractions of intact heart muscle could be reversed by increasing the extracellular concentration of Ca^{2+}. Subsequently, in mammalian cardiac muscle, Parmley & Braunwald (1967) found that propranolol at concentrations similar to those used in the present study (10^{-5} to 10^{-4} M) produced a substantial reduction (more than 20%) in the tension of cat papillary muscle evoked by electrical stimulation and a 30–40% fall in the rate of spontaneously beating cat right atria. More recently it has been shown that propranolol (10^{-4} M) can also decrease the action potential amplitude of guinea-pig atrial muscle partially depolarized by high external K^+ (Hashimoto *et al.*, 1979). These observations suggest that propranolol might impede the translocation or influx of Ca^{2+} from the external medium to the internal phase of the cardiac tissue. However, the question whether propranolol causes any inhibitory actions on Ca^{2+}-currents of mammlian cardiac muscle similar to those found in *Helix* neurones, requires further study.

The techniques described in this paper provide a method for examining effects of β-blockers on each single ionic current in the excitable cell membrane. This approach might well prove to be a useful method for electrophysiological studies of various compounds acting on the excitable cell membrane and also for use in primary screening tests for such compounds.

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(Received September 2, 1980
Revised January 28, 1981)