Br. J. Pharmac. (1981), **74**, 365–370

COMPARATIVE STUDIES OF (−)-, (±), (+)-PROPRANOLOL, ATENOLOL, GUANETHIDINE, BRETYLIUM AND TETRACAINE ON ADRENERGIC TRANSMISSION

M. KAIHO[1], T. KUBO & Y. MISU

Department of Pharmacology, Yokohama City University School of Medicine, Yokohama 232, Japan

1 Effects of (−)-, (±)-, and (+)-propranolol, atenolol, guanethidine, bretylium and tetracaine were studied on relaxation responses of rabbit ileum and contractile responses of rabbit pulmonary artery and guinea-pig vas deferens to electrical nerve stimulation (2 to 50 Hz).

2 In the ileum, inhibition by tetracaine 3.3×10^{-6} M occurred at high frequencies of stimulation, while bretylium 1.2×10^{-4} M and guanethidine 2×10^{-5} M inhibited responses at all frequencies, the latter producing greater inhibition at low frequencies.

3 (±)-Propranolol 10^{-5} M produced a tetracaine-type inhibition after 1 h and a bretylium-pattern after 2 h in the ilea and pulmonary arteries and a transition from bretylium- to guanethidine-pattern in the vas deferens, while atenolol 2×10^{-5} to 10^{-4} M produced guanethidine-type inhibition in all preparations.

4 (−)-, (±)-, and (+)-Propranolol 3×10^{-6} to 3.3×10^{-5} M were equipotent in the vas deferens and ileum. However, inhibition by (−)-propranolol 3.3×10^{-5} M persisted in the ileum, while that by the (+)-isomer was partially restored by washing.

5 (−)- or (+)-Propranolol 3.3×10^{-5} M or atenolol 2×10^{-5} M did not inhibit relaxation of the ileum after the bath temperature was maintained at 4°C for 2 h during drug application.

6 In conclusion, propranolol and atenolol both have gradually developing guanethidine-like adrenergic neurone blocking actions.

Introduction

The mechanism of the antihypertensive action of β-adrenoceptor blocking agents is not well understood. Relatively high doses of these antagonists inhibit adrenergic transmission in various sympathetic nerve-effector preparations without antagonizing responses to exogenously applied noradrenaline (Rand, Law, Story & McCulloch, 1976; Weinstock, 1976). However, it has not been established whether this presynaptic action is due to a simple local anaesthetic-like activity (Day, Owen & Warren, 1968; Barrett & Nunn, 1970; Hughes & Kneen, 1976) or to a so-called guanethidine-like adrenergic neurone blocking activity (Eliash & Weinstock, 1972; Mylecharane & Raper, 1973). Using three different isolated sympathetic nerve-effector preparations, we have attempted to differentiate the modes of action of propranolol and atenolol regarding modifications of frequency-response relationships. We compared guanethidine, bretylium and tetracaine, because guanethidine preferentially inhibits responses to lower frequencies (Boura & Green, 1965), while inhibition induced by local anaesthetics is greater at higher frequencies in various neurone systems (Bentley, 1966; Strichartz, 1973; Courtney, 1975; Yeh & Narahashi, 1976). A preliminary account of this work has already appeared (Misu, Kaiho, Ogawa & Kubo, 1980).

[1]Present address: Department of Anatomy, Yokohama City University School of Medicine, Yokohama 232, Japan.

Methods

Rabbit ileum

The preparation described by Finkleman (1930) was used. Male rabbits, weighing 1.5 to 2 kg, were bled from the carotid arteries. A segment of ileum approximately 1 cm long with the periarterial nerve intact was isolated and set up in a 50 ml organ bath containing McEwen's solution (1956), bubbled with 5% CO_2 in O_2. The solution had the following composition (mM): NaCl 129.9, KCl 5.6, $CaCl_2$ 1.6,

0007-1188/81/10 0365-06 $01.00

$NaHCO_3$ 25, NaH_2PO_4 0.95, sucrose 13.3 and glucose 11.1. The final pH was 7.4 and the temperature maintained at 37°C. The nerve was stimulated by means of a suction electrode at 4 min intervals for 45 s at frequencies of 2, 5, 10, 20 and 50 Hz and supramaximal voltage (6 to 14 V) with rectangular pulses of 5 ms duration generated by an electronic stimulator MSE-3R (Nihon Kohden). Resting tension was adjusted to 0.5 g and movements of the ileum were recorded isotonically on an ink-writing oscillograph (Sanei Rectigraph 8S). Responses were calculated as a % inhibition of average amplitude of spontaneous contractions during nerve stimulation compared with the average amplitude before stimulation. In some experiments the temperature of the fluid surrounding the organ bath was reduced to 4°C.

Rabbit pulmonary artery

A ring approximately 5 mm long of the distal end of the main pulmonary artery with the right recurrent cardiac nerve was isolated from male rabbits (Bevan, 1962) and set up in aerated Krebs bicarbonate solution at 37°C. The composition of the solution was as follows (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.18, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 11.1. The resting tension was 1.5 g and contractions of radial muscles were recorded isotonically. Nerve stimulation was via a suction electrode with pulses of 2 ms duration, 10 V at 2, 5, 10 and 20 Hz for 15 s at 4 min intervals.

Guinea-pig vas deferens

The hypogastric nerve-vas deferens was prepared according to Huković's method (1961) and was suspended in gassed McEwen's (1956) solution at 37°C. The resting tension was 0.5 g. The nerve was stimulated through a suction electrode 3 s every 2 min during the experimental period of 2 h at a fixed frequency of 5, 8 or 20 Hz using 2 ms pulse width and supramaximal voltage (4 to 6 V).

In all preparations, a stabilization period of 1 to 3 h was allowed before drug application. Drugs were added to the bathing solution and washing of preparations was performed by overflowing the bath with about 500 ml of fresh solution. Results are expressed as a % of control relaxation responses of the ileum and of the maximum tension of contractor responses of pulmonary artery and vas deferens to sympathetic nerve stimulation with each frequency before the drug application. Student's *t* test was used to evaluate the data.

Drugs used were (−)-, (±)-and (+)-propranolol hydrochloride and (±)-atenolol hydrochloride (ICI), guanethidine hemisulphate (CIBA-Geigy), bretylium tosylate (Wellcome), tetracaine hydrochloride (Kyorin) and (−)-noradrenaline hydrochloride (Sigma). Noradrenaline was dissolved in 0.06 N HCl and the other drugs in distilled water. The concentrations of drugs in the bath are expressed as M.

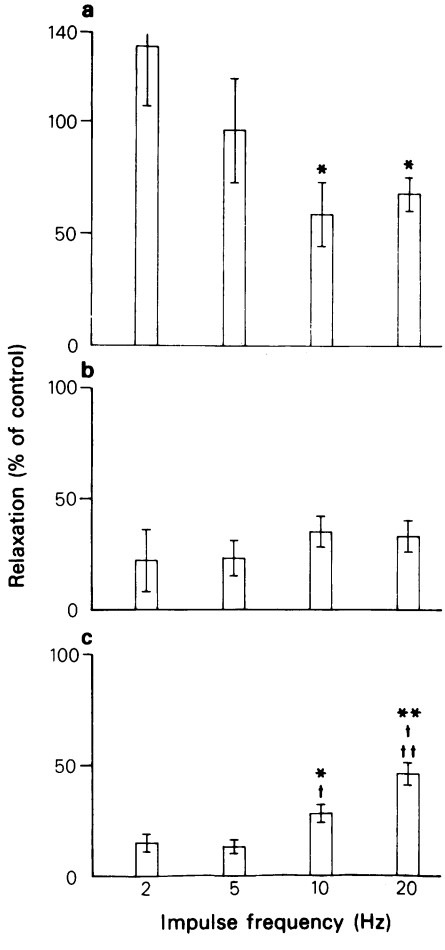

Figure 1 Effects of (a) tetracaine 3.3×10^{-6} M, (b) bretylium 1.2×10^{-4} M and (c) guanethidine 2×10^{-5} M on relaxation responses of rabbit isolated ileum to periarterial nerve stimulation. The nerve was electrically stimulated for 45 s at 4 min intervals with frequencies indicated on the abscissa scale. On the ordinate scale, responses to nerve stimulation with each frequency before drug application are taken as 100%. Stimulation was repeated 30 min after the addition of tetracaine and 30 min after washing preparations incubated with bretylium or guanethidine solution for 20 min. Vertical bars indicate s.e.mean from (a) 5, (b) 13 and (c) 25 observations. Statistical significance: $*P<0.05$, $**P<0.01$, compared to 2 Hz; $\dagger P<0.01$, compared to 5 Hz; $\dagger\dagger P<0.01$, compared to 10 Hz.

Results

Effects of tetracaine, bretylium, guanethidine, (±)-propranolol and atenolol on relaxation responses of rabbit ileum to periarterial nerve stimulation

A segment of the ileum suspended in McEwen's solution spontaneously contracted at the rate of 8 to 9/min after stabilization. Sympathetic nerve stimulation for 45 s at 4 min intervals produced a frequency-dependent inhibition of spontaneous contractions. Stimulation with 20 and 50 Hz produced almost total relaxation.

Tetracaine 3.3×10^{-6} M after 30 min inhibited relaxation responses to nerve stimulation only with higher frequencies (Figure 1). The inhibition was readily reversed when the preparations were washed with fresh solution. On the other hand, bretylium 1.2×10^{-4} M produced a frequency-independent inhibition and guanethidine 2×10^{-5} M inhibited all frequencies but with significantly greater effect at lower rates of stimulation. The effects of both adrenergic neurone blocking agents were not reversed by repeated washings.

(±)-Propranolol 10^{-5} M produced a gradual inhibition of the relaxation responses to nerve stimulation (Figure 2). The inhibition was significant only at the higher frequencies during the 1st hour ($P < 0.01$, compared with control relaxation response, respectively), but was effective against all frequencies after 2 h. At this time adding fresh solution restored the inhibition of responses to the higher frequencies: responses 2 h after the drug and 30 min after washing were 53.9 ± 8.0% and 94.0 ± 3.5% of control to 50 Hz nerve stimulation and 54.2 ± 11.7% and 52.5 ± 11.7% to 10 Hz, respectively ($n = 6$). Atenolol 2×10^{-5} M also gradually inhibited responses to nerve stimulation, but the inhibition was always more prominent at lower frequencies (Figure 2) and was not restored by washing. Inhibitory responses of the ileum to cumulatively applied noradrenaline 3×10^{-8}, 10^{-7} and 3×10^{-7} M were unaffected by the 10 min prior treatment with (±)-propranolol 10^{-5} M or atenolol 2×10^{-5} M.

Effects of (±)-propranolol and atenolol on contractor responses of rabbit pulmonary artery and guinea-pig vas deferens to sympathetic nerve stimulation

Contractor responses of both preparations to nerve stimulation were gradually impaired by (±)-propranolol 10^{-5} M and atenolol 10^{-4} M in a similar manner to that seen against relaxation responses of rabbit ileum.

In pulmonary arteries, (±)-propranolol-induced inhibition was greater at higher frequencies after 60 min but after 2 h was equal at all frequencies ($n = 3$). At this time washing with fresh solution

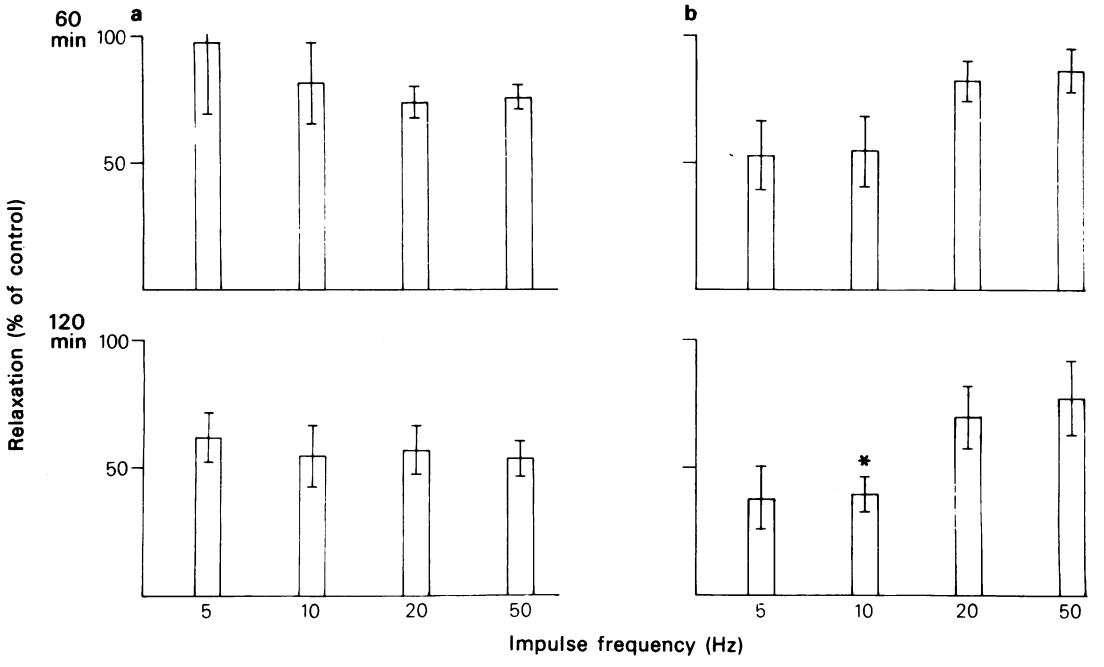

Figure 2 Effects of (a) (±)-propranolol 10^{-5} M and (b) atenolol 2×10^{-5} M on adrenergic transmission in the rabbit ileum. Statistical significance: $^*P < 0.05$, compared to 50 Hz. Vertical bars represent s.e.mean of 6 observations.

restored contractor responses at higher frequencies. The time course of inhibition by atenolol was slightly slower than that in rabbit ileum and the action was more prominent at lower frequencies after 2 h ($n = 5$). Washing produced no restoration.

In the vas deferens, 60 min exposure to (±)-propranolol produced similar inhibition at 5 and 20 Hz but the inhibition was significantly more marked at 5 Hz after 2 h (Figure 3). On the other hand, atenolol-induced inhibition was consistently greater against stimulation at 5 Hz.

Comparisons between pharmacological properties of (−)- and (+)-propranolol

In the guinea-pig vas deferens, (−)-, (±)-, and (+)-propranolol 10^{-5} M reduced contractor responses to 20 Hz nerve stimulation to 38.8 ± 6.8 ($n = 6$), 45.3 ± 4.0 ($n = 6$) and 46.2 ± 5.6% ($n = 6$) of control respectively 2 h after the application. In additional experiments, the concentrations of (−)- and (+)-propranolol were lowered to 5×10^{-6} M and 3×10^{-6} M and the stimulation frequency was reduced to 8 Hz. However, a significant discrepancy was not found between the inhibitory activities of the isomers.

After 2 h, there was no discrepancy between the inhibitory actions of (−)- (59.1 ± 5.2%, $n = 5$) and (+)- (48.2 ± 6.8%, $n = 9$) propranolol 3.3×10^{-5} M on relaxation responses of the rabbit ileum to 20 Hz nerve stimulation. The inhibition by (+)-propranolol unlike that by (−)-propranolol was partially restored by washing: relaxation response 30 min after washing was 52.2 ± 8.3% and 77.5 ± 8.5% of control for the (−)- and (+)-isomer, respectively.

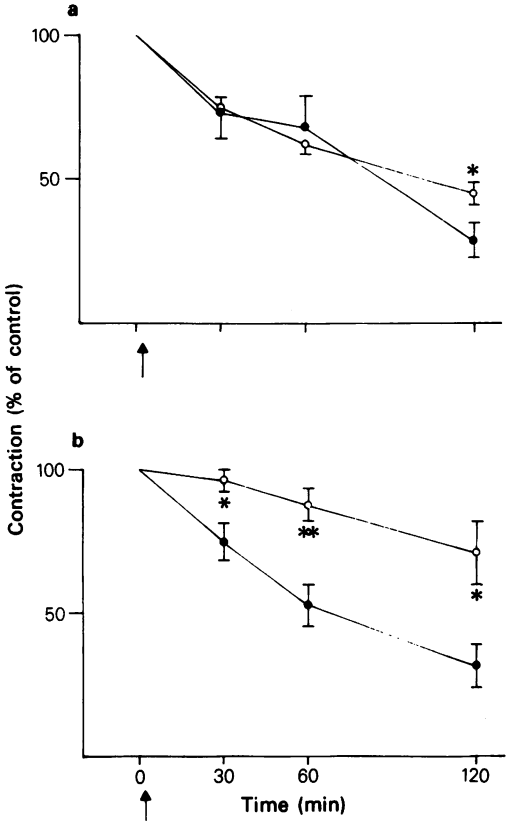

Figure 3 Time course of the effects of (a) (±)-propranolol 10^{-5} M and (b) atenolol 10^{-4} M on adrenergic transmission in guinea-pig isolated vas deferens. Hypogastric nerve was stimulated at 5 Hz (●) and 20 Hz (○) for 3 s at 2 min intervals. Abscissa scale shows time after drug application (at the arrows). Statistical significance: $*P < 0.05$, $**P < 0.01$, compared to 5 Hz. Vertical bars represent s.e.mean from at least 6 observations.

(−)- and (+)-Propranolol and atenolol at low temperature in rabbit ileum

At 37°C, relaxation response to 10 Hz nerve stimulation 2 h after atenolol 2×10^{-5} M and 30 min after washing was 34.2 ± 9.2% and 34.9 ± 14.9% ($n = 4$) of control, respectively.

After the temperature of organ bath had been reduced to 4°C during the drug application period of 2 h, (−)- and (+)-propranolol 3.3×10^{-5} M and atenolol 2×10^{-5} M produced no transmission failure: relaxation response 30 min after washing at 37°C was 97.2 ± 13.3% ($n = 5$), 101.1 ± 2.0% ($n = 9$) and 100.4 ± 1.0% ($n = 4$) of control, respectively.

Discussion

The present results demonstrate that the gradually developing adrenergic transmission failure induced by propranolol and atenolol is similar to a guanethidine-like neurone blocking action in the ileum and pulmonary artery of rabbits and guinea-pig vas deferens.

It was confirmed that in the ileum, bretylium inhibited responses to wide ranges of low to high frequencies and guanethidine produced a preferential inhibition of responses to lower frequencies (Boura & Green, 1965). On the other hand, the inhibitory action of tetracaine was greater at higher frequencies. This result is consistent with findings that inhibition by local anaesthetics is usually frequency-dependent in various neuronal systems (Bentley, 1966; Stricharz, 1973; Courtney, 1975; Yeh & Narahashi, 1976). These results suggest that the mode of action of adrenergic neurone blocking agents and local anaesthetics on adrenergic neurones may be different (Kubo & Misu, 1974; Misu, Nishio, Hosotani & Hamano, 1976; Maxwell & Wastila, 1977; Misu &

Nishio, 1978; Hosotani & Misu, 1978a; Hosotani & Misu, 1978b; Häusler & Haefely, 1979). The action of (±)-propranolol was biphasic with a gradual transition from tetracaine- to bretylium-like transmission failure in the ileum and pulmonary arteries and from a bretylium- to guanethidine-like pattern in the vas deferens. On the other hand, atenolol always produced a guanethidine-type failure in all preparations. This is consistent with the small membrane stabilizing activity of atenolol (Barrett, Carter, Fitzgerald, Hull & Le Count, 1973).

It was confirmed that tetracaine-induced inhibition was readily reversible, while bretylium- or guanethidine-induced failure could not be reversed by washing (Boura & Green, 1965; Rand & Wilson, 1967, Misu *et al.*, 1976). (±)-Propranolol (10^{-5} M)-induced failure in response to higher frequencies was readily reversible by washing and this observation is consistent with the findings of Day *et al.* (1968), Barrett & Nunn (1970) and Hughes & Kneen (1976), while that to lower frequencies was difficult to restore and is consistent with the findings of Mylecharane & Raper (1973). These results also suggest that propranolol has properties similar to those of tetracaine and guanethidine. The action of atenolol was irreversible and consistent with the properties of adrenergic neurone blocking agents.

Simple local anaesthetic activity of propranolol cannot be responsible for the antihypertensive action, because the latter action in man shows stereospecificity (Prichard, 1971; Rahn, Hawlina, Kersting & Planz, 1974; Waal-Manning, 1976) and the (−)- and (+)-isomers have equipotent membrane stabilizing actions (Barrett & Cullum, 1968). The guanethidine-like inhibitory actions of propranolol were not dissociable between (−)- or (±)- and (+)-isomers in the vas deferens and the ileum in the range of concentrations from 3×10^{-6} to 3.3×10^{-5} M in agreement with Mylecharane & Raper (1973) and Rand *et al.* (1976). However, failure induced by the highest concentration of (−)-propranolol remained after washing, while that by the (+)-isomer was partially reversed. This shows a dissociation between the pharmacological properties of the isomers of propranolol, suggesting that the guanethidine-like action of propranolol may be an additional factor in the antihypertensive effect.

If propranolol and atenolol do have guanethidine-like actions, they should be actively taken up into adrenergic nerve endings (Gulati & Jaykar, 1971; Hosotani & Misu, 1977; Hosotani & Misu, 1978b). In fact, a persistent transmission failure induced by propranolol and atenolol was prevented by exposure of the ileum to low temperature during the drug application period. Thus, the process of an active uptake of both β-adrenoceptor blocking agents is required for this failure. This result is in part consistent with findings that propranolol was markedly accumulated in cultured rat sympathetic ganglia and atria and that this accumulation was inhibited by imipramine but was not modified by ouabain (Saelens, Daniell & Webb, 1977). However, the present results show no difference between (−)- and (+)-propranolol.

We thank Ms K. Ogawa for technical assistance and M. Ohara, Kyushu University, for critical reading of the manuscript. Reprint requests to Y.M., please.

References

BARRETT, A.M., CARTER, J., FITZGERALD, J.D., HULL, R. & LE COUNT, D. (1973). A new type of cardioselective adrenoceptor blocking drug. *Br. J. Pharmac.*, **48**, 340P.

BARRETT, A.M. & CULLUM, V.A. (1968). The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. *Br. J. Pharmac.*, **34**, 43–55.

BARRETT, A.M. & NUNN, B. (1970). Adrenergic neuron blocking properties of (±)-propranolol and (+)-propranolol. *J. Pharm. Pharmac.*, **22**, 806–810.

BENTLEY, G.A. (1966). The effect of local anaesthetic and anti-adrenaline drugs on the response of sympathetically innervated smooth muscle preparations to electrical stimulation at different frequencies. *Br. J. Pharmac. Chemother.*, **27**, 64–80.

BEVAN, J.A. (1962). Some characteristics of the isolated sympathetic nerve-pulmonary artery preparation of the rabbit. *J. Pharmac. exp. Ther.*, **137**, 213–218.

BOURA, A.L.A. & GREEN, A.F. (1965). Adrenergic neurone blocking agents. *A. Rev. Pharmac.*, **5**, 183–212.

COURTNEY, K.R. (1975). Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA 968. *J. Pharmac. exp. Ther.*, **195**, 225–236.

DAY, M.D., OWEN, D.A.A. & WARREN, P.R. (1968). An adrenergic neuron blocking action of propranolol in isolated tissues. *J. Pharm. Pharmac.*, **20**, Suppl., 130S–134S.

ELIASH, S. & WEINSTOCK, M. (1972). Factors influencing the adrenergic neurone blocking action of propranolol. *Br. J. Pharmac.*, **45**, 630–634.

FINKLEMAN, B. (1930). On the nature of inhibition in the intestine. *J. Physiol.*, **70**, 145–157.

GULATI, O.D. & JAYKAR, S. (1971). Factors affecting the action of guanethidine on adrenergic neurones. *Br. J. Pharmac.*, **42**, 352–363.

HÄUSLER, G. & HAEFELY, W. (1979). Modification of release by adrenergic neuron blocking agents and agents that alter the action potential. In: *The Release of Catecholamines from Adrenergic Neurones.* ed. Paton, D.M. pp. 185–216, Oxford: Pergamon Press.

HOSOTANI, T. & MISU, Y. (1977). Sodium sensitive active transport of bretylium into adrenergic neurons for the development of blockade in rabbit ileum. *Archs int. Pharmacodyn.*, **226,** 235–245.

HOSOTANI, T. & MISU, Y. (1978a). Prevention by guanethidine analogues of output of noradrenaline induced by sodium reduction in rabbit ventricular slices. *Br. J. Pharmac.*, **64,** 37–45.

HOSOTANI, T. & MISU, Y. (1978b). Further evidence for the prevention by guanethidine of noradrenaline efflux from rabbit ventricular slices induced by a low external sodium concentration. *Br. J. Pharmac.*, **64,** 163–165.

HUGHES, I.E. & KNEEN, B. (1976). The effect of propranolol on sympathetic nerve stimulation in isolated vasa deferentia. *J. Pharm. Pharmac.*, **28,** 200–205.

HOKOVIĆ, S. (1961). Responses of the isolated sympathetic nerve-ductus deferens preparation of the guinea-pig. *Br. J. Pharmac. Chemother.*, **16,** 188–194.

KUBO, T. & MISU, Y. (1974). Mode of action of guanethidine on adrenergic neurons and its dependence on sodium. *Jap. J. Pharmac.*, **24,** 307–318.

MAXWELL, R.A. & WASTILA, W.B. (1977). Adrenergic neuron blocking drugs. In: *Handbook of Experimental Pharmacology, Volume 39, Antihypertensive Agents*, ed. Gross, F. pp. 161–261. Berlin, Heidelberg, New York: Springer-Verlag.

McEWEN, L.M. (1956). The effect on the isolated rabbit heart of vagal stimulation, and its modification by cocaine, hexamethonium and ouabain. *J. Physiol.*, **131,** 678–689.

MISU, Y., KAIHO, M., OGAWA, K. & KUBO, T. (1980). Actions of β-receptor blocking agents on adrenergic neurons. *Jap. J. Pharmac.*, **30,** Suppl., 21P.

MISU, Y. & NISHIO, H. (1978). Action of guanethidine on rabbit atrial membranes. *Br. J. Pharmac.*, **63,** 17–23.

MISU, Y., NISHIO, H., HOSOTANI, T. & HAMANO, S. (1976). A new guanidine derivative: dissociation of the adrenergic neuron blocking activity from local anesthetic activity. *Jap. J. Pharmac.*, **26,** 367–375.

MYLECHARANE, E.J. & RAPER, C. (1973). Further studies on the adrenergic neuron blocking activity of some β-adrenoceptor antagonists and guanethidine. *J. Pharm. Pharmac.*, **25,** 213–220.

PRICHARD, B.N.C. (1971). Aspects of the evaluation of antihypertensive drugs. *Archs int. Pharmacodyn.*, **192,** Suppl., 193–208.

RAHN, K.H., HAWLINA, A., KERSTING, F. & PLANZ, G. (1974). Studies on the antihypertensive action of the optical isomers of propranolol in man. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **286,** 319–323.

RAND, M.J., LAW, M., STORY, D.F. & McCULLOCH, M.W. (1976). Effects of β-adrenoceptor blocking drugs on adrenergic transmission. *Drugs*, **11,** Suppl., 1, 134–143.

RAND, M.J. & WILSON, J. (1967). The relationship between adrenergic neurone blocking activity and local anaesthetic activity in a series of guanidine derivatives. *Eur. J. Pharmac.*, **1,** 200–209.

SAELENS, D.A., DANIELL, H.B. & WEBB, J.G. (1977). Studies on the interactions of propranolol with adrenergic neurons. *J. Pharmac. exp. Ther.*, **202,** 635–645.

STRICHARTZ, G.R. (1973). The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. gen. Physiol.*, **62,** 37–57.

WAAL-MANNING, H.J. (1976). Hypertension: which beta-blocker? *Drugs*, **12,** 412–441.

WEINSTOCK, M. (1976). The presynaptic effect of β-adrenoceptor antagonists on noradrenergic neurones. *Life Sci.*, **19,** 1453–1466.

YEH, J.Z. & NARAHASHI, T. (1976). Frequency-dependent block of sodium channel in normal and pronase-treated squid axons. *Fedn. Proc.*, **35,** 846.

(Received December 10, 1980.
Revised June 18, 1981.)