Time-independent effects on cardiac action potential upstroke velocity (resting block) and lipid solubility of beta adrenergic blockers

H. Sada and T. Ban

Department of Pharmacology, School of Medicine, Yamaguchi University, 1144 Kogushi, Ube 755 (Japan), 30 June 1980

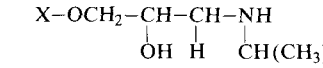
Summary. The depressant actions of 100 μM of 11 β-adrenergic blockers on the maximum upstroke volocity of action potential (V_{max}) in guinea-pig papillary muscles correlated well with the log n-octanol/phosphate buffer partition coefficients of these compounds.

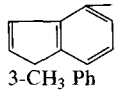
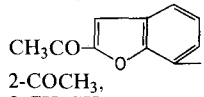
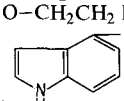
It has been demonstrated that an inhibition of the fast sodium current in nerve fibres by local anesthetics[1] as well as a reduction of the maximum upstroke velocity of cardiac action potential (V_{max}) by antiarrhythmics[2-4] is manifested in a rate-dependent manner. That is, the suppression of the Na system is progressively increased as the driving rate is raised, whereas only time- or rate-independent suppression of the Na system (resting block)[5,6] occurs at the lowest rates of stimulation. Such rate-dependent effects are assumed[5-7] to be derived from a time- and voltage-dependent interaction of drug molecules with the receptor sites within the sodium channels. The potency of a series of local anesthetics of the amide type for the resting block in nerves has been shown to correlate well with the lipid solubility of the drugs[5,6]. Drugs of this type, such as lidocaine, have been demonstrated by Gliklich and Hoffman[8] to act on the sodium channels in cardiac tissues from inside the cell. In order to see whether similar relationships hold in cardiac tissues, we investigated the effects of 11 β-adrenergic blocking agents on the $\dot{V}_{max}$ of action potentials of guinea-pig hearts at an extremely low rate of stimulation. All the β-blockers we used in the present study are aryloxyisopropylaminopropanol derivatives, as shown in the table.

Methods. By means of a conventional glass microelectrode technique, action potentials and their first time-derivatives ($\dot{V}_{max}$) were recorded in isolated guinea-pig papillary muscles. The muscles, mounted in an organ bath (1 ml volume), were superfused at a rate of 4 ml/min with a modified Tyrode solution (5.4 mM KCl and 10.0 mM glucose), gassed with 95% O_2 + 5% CO_2 (37 °C, pH 7.2–7.4). The preparations were continuously driven at a rate of 1/37 sec. In our preliminary studies, this rate was found to be sufficiently long to ensure the uppermost recoveries of the time-dependent suppression of $\dot{V}_{max}$ and therefore to retain only the resting block for all drugs used here. The extent of this block is expressed in terms of $\dot{V}_{max}$ values obtained after 30, 45 and 60 min of exposure to either one of the drugs (100 μM), relative to that immediately before exposure. The n-octanol/buffer partition coefficient (P) for each drug was determined at pH 7.4 according to Hellenbrecht et al.[9]. The details have been given in our previous paper[10]. Its approximate value, π[11,12], a substituent constant having a property of additivity, was calculated according to Tute[11] and Hansch et al.[12].

Results and discussion. As can be seen in the table, the potency of these drugs for the resting block was increased in the increasing order of log P of the drugs ($r = 0.924$, $n = 11$, $P < 0.001$; at 30 min) or the π value, whereas it did not correlate well with the molecular weight of the drugs ($r = 0.424$, $n = 11$, $P > 0.2$; at 30 min). The fit became poorer

The relation of the lipophilicity of the β-blockers to the levels of resting block



Drug[a]	Molecular weight	X[b]	Lipophilicity		Resting block (%)[e]		
			log P[c]	π[d]	30 min	45 min	60 min
Propranolol	259.3	Naphthyl-	1.211	1.32	n = 5 30.2 ± 4.7	n = 5 22.9 ± 4.1	n = 5 15.3 ± 3.3
Alprenolol	249.3	2-CH_2=CH-H_2C Ph	0.995	1.10	n = 6 51.9 ± 4.9	n = 6 46.9 ± 4.4	n = 6 42.2 ± 3.3
Kö 707	237.3	3,5-CH_3 Ph	0.726	1.12	n = 6 53.9 ± 4.9	n = 6 57.6 ± 6.2	n = 3 55.7 ± 8.6
Indenolol	247.3		0.702	-	n = 5 52.7 ± 8.5	n = 5 46.7 ± 9.0	n = 4 42.2 ± 3.3
Kö 592	223.3	3-CH_3 Ph	0.223	0.56	n = 6 73.3 ± 4.3	n = 5 69.6 ± 6.3	n = 5 70.6 ± 7.3
Oxprenolol	265.3	2-OCH_2-CH=CH_2 Ph	0.118	0.46	n = 6 71.8 ± 4.2	n = 5 72.2 ± 5.4	n = 5 74.7 ± 4.8
Befunolol	275.3		−0.076	-	n = 6 89.4 ± 3.0	n = 6 90.3 ± 5.4	n = 6 87.7 ± 6.8
Acebutolol	336.4	2-$COCH_3$, 3-$CH_3CH_2CH_2CONH$ Ph	−0.276	−0.49	n = 5 92.8 ± 1.1	n = 5 90.7 ± 2.9	n = 4 91.9 ± 2.1
Metoprolol	267.4	4-CH_3-O-CH_2CH_2 Ph	−0.310	-	n = 5 90.1 ± 1.3	n = 5 94.2 ± 2.7	n = 4 93.8 ± 2.2
Pindolol	248.3		−0.328	0.01	n = 5 85.4 ± 2.3	n = 5 86.1 ± 3.4	n = 5 80.9 ± 3.8
Kö 1313	234.3	2-CN Ph	−0.481	−0.57	n = 5 75.0 ± 5.2	n = 5 75.5 ± 5.4	n = 3 69.2 ± 5.0

[a] All drugs used were hydrochloride salts except for metoprolol (tartarate) and pindolol (free base) which was dissolved in equimolar concentrations of tartaric acid. Molecular weight is calculated for the free-form of a drug; [b] X represents aryl group (Ph = phenyl); [c] log n-octanol/phosphate buffer partition coefficient. [d] Calculated according to Tute[11] and Hansch et al.[12] [e] Resting block is defined here as the $\dot{V}_{max}$ after, relative to that before drug addition (expressed as percent).

after 60 min of exposure, since the effects of highly lipophilic drugs such as propranolol and alprenolol became more prominent and deviated from the regression line. On the basis of 'modulated receptor hypothesis' in nerves[5-7], the resting block in the present experiments may be interpreted in the following 2 ways; a) only a fraction of the drug molecules is diffused away, during a long pause in stimulation, from the binding sites; this may have some bearing on the gating mechanism of the Na channels; and/or b) they associate rapidly with the sites at the moment of an initiation of action potential despite a complete loss of drug molecules at the receptor site during a pause, which may be the case with lidocaine[5] ('apparent' tonic block). At present, it can not be decided which mechanism plays a greater role. It is well known that hydrophilic β-blockers, such as sotalol, practolol and INPEA[13], and atenolol[14] have only weak or no quinidine-like activity. Sada et al.[15] demonstrated that the order of potency for the depressant effects of several β-blockers on $\dot{V}_{max}$, evaluated in the myocardium at 1 Hz, did not run completely in parallel with the lipophilic properties of the drugs. These results suggest that the time-independent effects of these drugs are determined mainly by the lipophilic property of the drug molecules in cardiac tissues, as is the case with nerve fibres[6,7], whereas these effects are more-or-less modified by the rate-dependent effects at the rate of 1 Hz or those rates at which the latter effects were operative. Finally, we must point out the possibility that these drugs may not affect the sodium channels directly but affect some other currents, by which the sodium channel is secondarily affected[16].

1 K.R. Courtney, J.J. Kendig and E.N. Cohen, Anesthesiology *48*, 111 (1978).
2 L.M. Hondeghem and B.G. Katzung, Biochim. biophys. Acta *472*, 373 (1977).
3 T. Ban, Jap. J. Pharmac. *27*, 865 (1977).
4 H. Sada, Naunyn-Schmiedebergs Arch. Pharmac. *304*, 191 (1978).
5 K.R. Courtney, J.J. Kendig and E.N. Cohen, J. Pharmac. exp. Ther. *207*, 594 (1978).
6 K.R. Courtney, J. Pharmac. exp. Ther. *213*, 114 (1980).
7 B. Hille, J. gen. Physiol. *69*, 497 (1977).
8 J.I. Gliklich and B.F. Hoffman, Circulation Res. *43*, 638 (1978).
9 D. Hellenbrecht, B. Lemmer, G. Wiethold and H. Grobecker, Naunyn-Schmiedebergs Arch. Pharmac. *277*, 211 (1973).
10 S. Harada, T. Ban, T. Fujita and A. Koshiro, submitted.
11 M.S. Tute, Adv. Drug. Res. *6*, 1 (1971).
12 C. Hansch, A. Leo, S.H. Unger, H. Kim, D. Nikaitani and J. Alien, J. med. Chem. *16*, 1207 (1973).
13 J.D. Fitzgerald, Clin. Pharmac. Ther. *10*, 292 (1969).
14 A.M. Barrett, J. Carter, J.D. Fitzgerald, R. Hull and D.L. Count, Br. J. Pharmac. *48*, 340 (1973).
15 H. Sada, T. Ban and M. Kojima, Jap. J. Pharmac. *29*, suppl. 157 (1979).
16 I.R. Cohen and G.R. Strichartz, Biophys. J. *17*, 275 (1977).

Direct excitant action of convulsant barbiturates[1]

P.R. Andrews[2], R.H. Evans[3], G.A.R. Johnston[4] and M. Willow

Departments of Physical Biochemistry and Pharmacology, John Curtin School of Medical Research, Australian National University, P.O. Box 334, A.C.T. 2601 (Australia), 8 February 1980

Summary. 5-(2-cyclohexylideneethyl)-5-ethylbarbituric acid (CHEB) and other convulsant barbiturates produced depolarization of isolated dorsal root fibres and other unmyelinated nerves from the rat. CHEB-induced depolarizations, unlike kainate-induced depolarizations were abolished by omission of Ca^{2+} from, or addition of ruthenium red (5 µM) to, the bathing medium.

The action of depressant barbiturate drugs is produced largely through potentiation[5-7] and/or mimicry[8,9] of the effects of the inhibitory neurotransmitter γ-aminobutyric acid (GABA)[10]. However, some barbiturate derivatives have a convulsant action[11] which is unlikely to be mediated by the same mechanisms that give rise to depressant action. The convulsant 5-(2-cyclohexylideneethyl)-5-ethyl barbituric acid (CHEB) has been reported to directly depolarize dorsal root ganglion cells, in the cat, thereby evoking spontaneous discharge in the peripheral ends of cut dorsal roots[12].

In the present experiments a series of barbiturate and non-barbiturate convulsants have been examined for their ability to depolarize isolated dorsal root fibres from the rat. Only barbiturates with convulsant activity in vivo produced depolarization of dorsal root fibres and other unmyelinated nerves. Furthermore, depolarizations of dorsal root fibres induced by CHEB, unlike kainate-induced depolarizations, were abolished by omission of Ca^{2+} from, or addition of ruthenium red (5 µM) to, the bathing medium. It is suggested that the convulsant barbiturates represent a hitherto undescribed class of excitants which depolarize cell membranes through a calcium-dependent mechanism.

The method of recording electrical polarity of spinal roots and other nerves, the composition of the bathing medium and the method of application of drugs have been described previously[13]. The action of convulsant barbiturates on motoneurones, as recorded from ventral roots, of hemisected spinal cords of immature (2–9-day-old) rats was similar to that of kainic acid[14] (figure, a). However, an action of these convulsants different from that of excitant amino acids is indicated because CHEB and other convulsant barbiturates were found to depolarize ventral roots isolated from immature rats and vagus nerve, sympathetic nerve and dorsal roots of mature rats; all of these being preparations which are not depolarized by excitant amino acids[15,16]. A phrenic nerve and ventral root from a mature rat were unaffected by CHEB (500 µM). The threshold concentration for the depolarization of sympathetic nerves (superior cervical pre- and post-ganglionic trunks) and dorsal roots of mature rats was relatively high (200 µM CHEB) while that for the vagus nerve was lower (25 µM CHEB). The preparation most sensitive to the depolarizing action of these compounds (threshold 2.5 µM CHEB) was the dorsal root of the immature rat (figure, b), consequently this preparation was