J. Pharm. Pharmacol. 1983, 35: 590–592
Communicated January 27, 1983

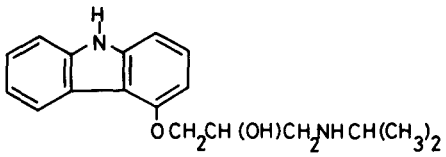
COMMUNICATIONS

A comparison of the selectivity of carazolol with that of other β_2-selective adrenoceptor antagonists

B. COSTIN, STELLA R. O'DONNELL*, JANET C. WANSTALL, *Pharmacology Section, Department of Physiology and Pharmacology, University of Queensland, Brisbane, 4067, Australia*

Adrenoceptor antagonists with high selectivity for β_2-adrenoceptors are used to characterize β-adrenoceptor populations in tissues, in both pharmacological and radioligand binding studies. The most widely used β_2-selective antagonists are ICI 118, 551 [erythro-DL-(7-methylindan-4-yloxy-3-isopropylamino-butan-2-ol], the marked β_2-selectivity of which is not in dispute (Bilski et al 1980; O'Donnell & Wanstall 1980; Dickinson & Nahorski 1981; Charlton et al 1982) and IPS 339 [(t-butylamino-3-ol-2-propyl)oximino-9-fluorene], for which the magnitude of the β_2-selectivity remains controversial (Imbs et al 1977; Minneman et al 1979; O'Donnell & Walduck 1981). Carazolol (I) is another

I



β-adrenoceptor antagonist for which there are conflicting conclusions concerning its β_2-selectivity (Lemoine & Kaumann 1978; Innis et al 1979; Cohen et al 1980). The β_2-selectivity of carazolol has been examined in the present study, and the results compared with results for other antagonists obtained in this laboratory under the same experimental conditions. Its β_2-selectivity has been determined from data on two tissues (guinea-pig trachea and atria, as described by O'Donnell & Wanstall 1979) and from data on only one tissue (guinea-pig trachea). Both methods employed fenoterol as a β_2-selective agonist and noradrenaline as a β_1-selective agonist. A method involving data from only one tissue has been advocated by Cohen et al (1980) since it avoids potential errors in the results due to differences in diffusional barriers to the receptor biophase which may occur in two different tissues.

Methods

Intrinsic tone tracheal chain preparations and spontaneously beating atrial preparations, taken from female guinea-pigs (300–600 g) pretreated with reserpine (1 mg kg^{-1} i.p. 18–24 h before the experiment), were set up as described by O'Donnell & Wanstall (1979). Experiments were carried out at 37 °C in Krebs solution aerated with 95% O_2, 5% CO_2. α-Adrenoceptors and neuronal and extraneuronal uptake mechanisms were blocked by treating the tissues with phenoxybenzamine (50 μM for 30 min followed by thorough washing of the tissue in phenoxybenzamine-free Krebs solution). Tracheal relaxation and atrial rate were recorded as described by O'Donnell & Wanstall (1979).

Agonist concentration-response curves were obtained in the absence and presence of increasing concentrations of carazolol, and the agonist EC50 (concentration causing 50% of the maximum response) interpolated. One or two different carazolol concentrations ([B]) were tested on any one preparation and the carazolol contact time was either 60 min or 90 min (see Results). The agonist EC50 in the presence of carazolol was divided by the agonist EC50 in the absence of carazolol to obtain concentration-ratio (CR) values. These were then corrected for spontaneous changes in the sensitivity of the preparations and used to obtain plots, referred to as Schild plots, of (log(CR-1) versus log[B], as described by O'Donnell & Wanstall (1979). Because Schild plots were obtained with slopes >1·0, (a) the pA_2 values quoted are those obtained by extrapolation of the Schild plots to log (CR-1) = 0, and (b) selectivity values quoted for carazolol are expressed as the antilog value of the average log unit separation of two appropriate Schild plots which did not differ significantly from one another in slope (O'Donnell & Walduck 1981).

The following carazolol concentrations were used at each of the contact times: *with noradrenaline as agonist* (trachea and atria)—60 min contact time, 1, 3, 10 or 30 nM; 90 min contact time, 1 or 30 nM; *with fenoterol as agonist* (trachea)—60 min contact time, 0·3, 1, 3 or 10 nM; 90 min contact time, 0·3 or 10 nM.

Drugs used: Carazolol (Boehringer Mannheim); fenoterol hydrobromide (Boehringer Ingelheim Ltd); noradrenaline acid tartrate (Sigma); phenoxybenzamine hydrochloride (Smith, Kline & French); reserpine (Serpasil, Ciba).

* Correspondence.

Table 1. Slopes of Schild plots ± s.e. and pA_2 values for carazolol on guinea-pig trachea and atria using noradrenaline or fenoterol as agonists and two different equilibration times (60 and 90 min) for carazolol.

Tissue	Agonist	Slope of Schild plot ± s.e. 60 min[a]	Slope of Schild plot ± s.e. 90 min[b]	pA_2[d] 60 min[a]	pA_2[d] 90 min[b]
Trachea	Fenoterol	1·65 ± 0·14*** (10, 16)[c]	1·63 ± 0·03*** (6, 6)	10·00	10·07
Trachea	Noradrenaline	1·37 ± 0·10** (9, 17)	1·21 ± 0·06* (6, 6)	9·46	9·78
Atria	Noradrenaline	1·34 ± 0·13* (8, 16)	1·35 ± 0·10* (5, 9)	9·59	9·66

[a] Data obtained at 4 different carazolol concentrations (see Methods).
[b] Data obtained at 2 different carazolol concentrations (the highest and lowest concentrations used in the 60 min contact time experiments).
[c] Number of animals, number of data points.
[d] pA_2 obtained by extrapolation of the Schild plot to log (CR-1) = 0.
Asterisks—slopes significantly greater than 1·0 * $0·05 > P > 0·01$, ** $0·01 > P > 0·001$, *** $P < 0·001$.

All drugs were obtained as pure powders except for reserpine which was obtained as a solution in ampoules. Stock solutions (100 mM) of fenoterol and noradrenaline were made up in 10 mM HCl. Stock solutions of carazolol (10 mM) and phenoxybenzamine (100 mM) were made up in absolute ethanol containing 10 mM HCl. Dilutions of all drugs were made in Krebs solution and kept on ice during the course of the experiment.

The composition of the Krebs solution was (mM): NaCl 114; KCl 4·7; $CaCl_2$ 2·5; KH_2PO_4 1·2; $MgSO_4$ 1·2; $NaHCO_3$ 25; glucose 11·7; ascorbic acid 1·1.

Results

Carazolol (60 or 90 min contact time) caused a parallel shift in the concentration-response curves to noradrenaline (atria and trachea) and fenoterol (trachea). The slopes of all the Schild plots for carazolol were significantly greater than 1·0 (Table 1). At the two different contact times, there were no significant differences between the slope values and no differences in pA_2 values for corresponding plots (Table 1).

Carazolol was more potent in antagonizing responses mediated by β_2-adrenoceptors than those mediated by β_1-adrenoceptors (pA_2 on trachea with fenoterol as agonist $> pA_2$ on atria with noradrenaline as agonist, Table 1). Using the data for 60 min contact time, the β_2:β_1-selectivity of carazolol was estimated in two ways *viz* either from the Schild plots for trachea, using fenoterol as agonist, and for atria, using noradrenaline as agonist (referred to as the two-tissue method), or from two Schild plots on trachea, using fenoterol and noradrenaline as agonists (referred to as the one-tissue method). The β_2:β_1-selectivity values obtained for carazolol were 4·6 (two-tissue method) and 5·6 (one-tissue method).

Discussion

Estimation of the β_2:β_1-selectivity of carazolol was complicated when, in experiments in which the contact time with carazolol was 60 min, the Schild plots, on both trachea and atria, were found to have slope values greater than 1·0. Since neuronal and extraneuronal uptake (agonist removal processes) were blocked in the experiments, it was assumed that the steep slopes were not due to block of the removal processes for the agonist by low concentrations of the antagonist (Furchgott 1972). However, it was possible that the time (60 min) allowed for equilibration of the carazolol was too short. This would have resulted in either a steep slope or an underestimated pA_2 value, depending on whether it was the antagonist-receptor interaction or diffusion of the antagonist into the receptor biophase respectively, which was rate-limiting (Kenakin 1980). An equilibration time of 60 min had been used by Cohen et al (1980; rat jugular vein preparations) although others have used equilibration times as long as 150 min (Kaumann et al 1979; rat atrial preparations). Additional experiments were carried out in the present study using a 90 min contact time with carazolol, but increasing the contact time had no effect on the slopes of the Schild plots or on the pA_2 values for carazolol. Thus, the results for 60 min contact time with carazolol have been used, since this is the time used to obtain data for other selective antagonists in this laboratory.

Carazolol was found to have a β_2:β_1-selectivity value of 4·6 by the two-tissue method and 5·6 by the one-tissue method, i.e. both methods gave the same estimate of selectivity and a value comparable to that obtained by Cohen et al (1980) on rat jugular vein using a one-tissue method. These values are all much less than the value of 30 quoted by Lemoine & Kaumann (1978), using data on trachea and heart, and the reasons for this discrepancy are not clear.

Table 2 summarizes the β_2-pA_2 values and β_2:β_1-selectivity values for some β_2-selective antagonists which have been studied in this laboratory. The values for selectivity have been calculated by both the two-tissue and the one-tissue methods. Carazolol has the highest potency on β_2-adrenoceptors, but compared with the other drugs, its β_2:β_1-selectivity is low. Table 2 also demonstrates, for other β_2-selective antagonists as well as for carazolol, that there was only a 1·2 fold difference between the estimates of selectivity obtained

Table 2. Potency and selectivity of β_2-selective adrenoceptor antagonists.

Antagonist	pA_2 on guinea-pig trachea (fenoterol as agonist) 'β_2-pA_2'	β_2:β_1-selectivity value Two-tissue method[a]	One-tissue method[b]
ICI 118,551[c]	8·69 ± 0·05 (7)[e]	53·7	43·7
Butoxamine[c]	6·38 ± 0·08 (6)	17·0	13·8
H35/25[c]	6·64 ± 0·07 (8)	13·5	16·2
α-Methyl-propranolol[c]	8·54 ± 0·11 (8)	11·0	13·8
Carazolol[d]	10·0	4·6	5·6
IPS 339[d]	7·54	3·3	—

[a] Selectivity calculated from data on guinea-pig trachea and guinea-pig atria.
[b] Selectivity calculated from data obtained only on guinea-pig trachea.
[c] pA_2 values and selectivity values by the two tissue method are from O'Donnell & Wanstall (1979 or 1980). These selectivity values represent antilog [mean pA_2 trachea (fenoterol as agonist) − mean pA_2 atria (noradrenaline as agonist)]. Selectivity values by the one tissue method represent antilog [mean pA_2 trachea (fenoterol as agonist) − means pA_2 trachea (noradrenaline as agonist)] using pA_2 values from O'Donnell & Wanstall (1979 or 1980).
[d] pA_2 values for carazolol (this paper) and for IPS 339 (O'Donnell & Walduck 1981) were obtained by extrapolation of the Schild plots to log (CR-1) = 0 because the slopes of these plots were significantly >1·0. The selectivity values represent the antilog of the average log unit separation between the Schild plots on trachea (fenoterol as agonist) and atria (noradrenaline as agonist) or only on trachea (fenoterol and noradrenaline as agonists).
[e] Number of animals.

by the two- or one-tissue (using selective agonists) methods, respectively. This supports the view expressed by Cohen et al (1980) that the selectivity value for an antagonist can be obtained on one tissue, provided that the response of that tissue can be mediated by both β_1- and β_2-adrenoceptors. The only antagonist for which we found some discrepancy between its selectivity values by the two methods, was atenolol. This antagonist is β_1-selective and the β_1:β_2-selectivity values obtained were 27·5 by the two-tissue method (O'Donnell & Wanstall 1979) and 13·2 by the one-tissue method (calculated from the trachea data of O'Donnell & Wanstall 1979) i.e. the two values differed by a factor of 2. Since the predominant adrenoceptor sub-type in guinea-pig trachea is β_2, this observation could suggest that the one-tissue method may be most accurate when the predominant receptor sub-type in the tissue is that for which the antagonist is selective. More data for other β_1-selective antagonists is needed to clarify this point.

In conclusion, carazolol has high potency as an antagonist at β_2-adrenoceptors but its β_2:β_1-selectivity is modest compared with other drugs. This study has also confirmed, using data for carazolol and other β_2-selective antagonists, that, at least for β_2-selective antagonists and for guinea-pig trachea (in which relaxation responses are mediated by β_1- and β_2-adrenoceptors, but with β_2 being predominant), selectivity values obtained on a single tissue (from Schild plots using fenoterol and noradrenaline as agonist) are the same as those obtained by the trachea-atria method described by O'Donnell & Wanstall (1979).

This work was supported by a grant from the National Health and Medical Research Council of Australia and this aid is gratefully acknowledged. One of us (JCW) is a NH & MRC Senior Research Officer. We would like to thank Miss Jane Baxter for her excellent technical assistance.

REFERENCES

Bilski, A., Dorried, S., Fitzgerald, J. D., Jessup, R., Tucker, H., Wale, J. (1980) Br. J. Pharmacol. 69: 292P

Charlton, K. G., Martorana, M. G., Rodger, I. W., Shahid, M. (1982) Ibid. 76: 259P

Cohen, M. L., Ruffolo, R. R., Wiley, K. S. (1980) J. Pharmacol. Exp. Ther. 215: 325–331

Dickinson, K. E. J., Nahorski, S. R. (1981) Eur. J. Pharmacol. 94: 43–52

Furchgott, R. F. (1972) in: Blaschko, H., Muscholl, E. (eds) Handbook of Experimental Pharmacology. Springer, New York, 33, pp 283–335

Imbs, J. L., Miesch, F., Schwartz, J., Velly, J., Leclerc, G., Mann, A., Wermuth, C. G. (1977) Br. J. Pharmacol. 60: 357–362

Innis, R. B., Correa, F. M. A., Snyder, S. H. (1979) Life Sci. 24: 2255–2264

Kaumann, A. J., Morris, T. H., Birnbaumer, L. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 307: 1–8

Kenakin, T. P. (1980) Eur. J. Pharmacol. 66: 295–306

Lemoine, H., Kaumann, A. J. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 302: R53

Minneman, K. P., Hedberg, A., Molinoff, P. B. (1979) J. Pharmacol. Exp. Ther. 211: 502–508

O'Donnell, S. R., Walduck, K. (1981) J. Pharm. Pharmacol. 33: 223–225

O'Donnell, S. R., Wanstall, J. C. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 308: 183–190

O'Donnell, S. R., Wanstall, J. C. (1980) Life Sci. 27: 671–677