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Comparison of β-adrenoceptor populations in cat and guinea-pig left atria

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The subtypes of β-adrenoceptor present in left atrial preparations from the guinea-pig and cat have been assessed using both responses obtained in organ bath experiments and radioligand binding studies. From the positive inotropic responses to procaterol, the pK_B values for practolol using a variety of agonists, and from displacement of [^{125}I]cyanopindolol from left atrial membrane homogenates by the selective β_1- and β_2-adrenoceptor antagonists L643,717-01J10 and ICI 118,551, it was concluded that guinea-pig left atria possess only β_1-adrenoceptors, whilst cat left atria possess both β_1- and β_2-adrenoceptor subtypes.

Positive inotropic responses to sympathomimetic drugs in left atrial preparations result from activation of β-adrenoceptors located throughout the tissue, and one atrium yields a sufficient quantity of membrane homogenate for receptor binding experiments with [^{125}I]cyanopindolol. Thus, in general, left atrial preparations are well-suited for correlating organ bath responses with results from radio ligand binding studies.

Previous studies have indicated that inotropic responses in both guinea-pig and cat left atria result from the activation of homogeneous populations of β_1-adrenoceptors within the tissues (Vlietstra & Blinks 1976; Kaumann et al 1978; Zaagsma et al 1979; McPherson et al 1984). However, more recent studies utilizing the highly selective β_2-adrenoceptor agonist procaterol, have led to the suggestion that both β_1- and β_2-adrenoceptors may be involved in the inotropic actions in the two species (Johansson & Persson 1983; Kaumann et al 1983).

In the present study we have sought to clarify the divergent results utilizing both organ bath experiments in which inotropic activity in left atrial preparations from guinea-pigs and cats has been assessed, and radioligand binding studies using membrane preparations of the same tissues.

METHODS

Left atria were taken from reserpine-pretreated guinea-pigs (1 mg kg^{-1}, i.p. 18 h, 200–500 g) and α-chloralose (80 mg kg^{-1}, i.p.) anaesthetized cats (0·25 mg kg^{-1}, i.p. 18 h, 400–1000 g: age >8 weeks). Pretreatment of both species with reserpine insured that responses obtained to exogenous agonists and their interaction with selected antagonists, were not complicated by concurrent release of endogenous neurotransmitter from sympathetic nerve terminals. Preparations, which were bathed at 37 °C in Krebs solution (NaCl 118, KCl 4·7, $CaCl_2$ 1·9, $NaHCO_3$ 25, $MgSO_4$ 1·2, glucose 11·7, NaH_2PO_4 1·2; EDTA 0·1, ascorbic acid 0·1 mM) gassed with 5% CO_2 in O_2, were electrically driven at 2·5 Hz using pulses of 1 ms duration at twice threshold driving voltage (range 1–3 V). Neuronal and extraneuronal uptake were inhibited by either pretreating tissues with phenoxybenzamine (50 μM, 30 min incubation, followed by 6 washes in 30 min) or by the addition of desipramine (1 μM) and hydrocortisone (50 μM) to the bathing solution. Changes in tension were recorded using a Grass FT03c transducer coupled to a Grass polygraph. In the experiments in which the effects of procaterol were studied, constant cumulative concentration-effect curves were first established to (−)-isoprenaline followed by a curve for procaterol.

In other experiments, concentration-effect curves were established to (−)-noradrenaline, (−)-adrenaline, (−)-isoprenaline and (±)-*N*-*t*-butylnoradrenaline before and after a 30 min incubation period with practolol (5 μM). Agonist EC50 values in the absence and presence of antagonists were used to calculate pK_B values ($-\log K_B$, Furchgott 1972).

In radioligand binding studies (see McPherson et al 1984 for full description), left atria were taken from non-reserpine-pretreated guinea-pigs and cats, and homogenized in ice-cold Krebs phosphate buffer (NaCl 119, KCl 4·8, $MgSO_4$ 1·2, $CaCl_2$ 1·9, glucose 11·7, NaH_2PO_4 1·3, Na_2HPO_4 8·7 mM, pH 7·4). Following centrifugation, membrane pellets were resuspended in 300 vol. buffer. Triplicate assays were performed in disposable polystyrene tubes in which 150 μl of homogenate was combined with 100 μl Krebs phosphate buffer containing the radio-

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ligand [^{125}I]cyanopindolol ([^{125}I]CYP; 10–200 pM saturation studies; 50–80 pM displacement studies), guanosine triphosphate (GTP) (0·1 mM), ascorbic acid (1 mM), EDTA (0·1 mM) and competing drug. Assays were terminated by rapid filtration after a 70 min incubation period at 37 °C. Specific binding was taken as the difference between total binding and that in the presence of propranolol (1 μM). Saturation and drug displacement data were analysed using two computer programs, EBDA (McPherson 1983a, b) which performed preliminary Scatchard, Hill and Hofstee analyses and created a file for LIGAND (Munson & Rodbard 1980), which was used to obtain final parameter estimates. The drugs used were (−)-isoprenaline bitartrate (Wyeth); procaterol hydrochloride (Warner Lambert); guanosine triphosphate (GTP), (−)-noradrenaline hydrochloride, (−)-adrenaline bitartrate (Sigma); (±)-*N*-*t*-butylnoradrenaline methansulfonate (Sterling Winthrop); practolol and ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) hydrochloride (Imperial Chemical Industries); desipramine hydrochloride and reserpine (Serpasil, Ciba-Geigy); L643,717-01J10 [(*S*)-2(P-[3-(3,4-dimethoxyphenethylamino)-2-hydroxypropoxy]-phenyl)-4-(2-thienyl)imidazole dihydrochloride] (Merck, Sharp & Dohme); phenoxybenzamine hydrochloride (Smith, Kline & French) and hydrocortisone sodium succinate (Glaxo). Stock solutions (10 mM) of drugs were prepared in either 10 mM HCl or distilled water. Phenoxybenzamine (0·1 M) was dissolved in acidified (1 μl 10 M HCl ml^{-1}) ethanol (95%). Dilutions were made using Krebs solution containing 1 mM ascorbic acid.

RESULTS

(−)-Isoprenaline elicited positive inotropic effects in guinea-pig and cat isolated left atrial preparations, mean pD_2 values of 8·4 ± 0·1 (n = 4) and 9·9 ± 0·1 (n = 4) respectively being obtained in the two tissues. While procaterol (1 nM–30 μM) was close to a full agonist (94 ± 5% (n = 4) of (−)-isoprenaline maximum response) and had a mean pD_2 value of 6·93 ± 0·14 (n = 4) in cat left atria, the drug produced only threshold positive inotropic responses in guinea-pig left atria (<5% of maximum response (−)-isoprenaline, n = 4) over the same concentration range. Fig. 1 shows mean concentration-effect curves for the two agonists in cat atrial preparations under control conditions and responses to procaterol after 40 min incubation with ICI 118,551 (0·1 μM). It is evident that ICI 118,551 produced a rightward shift of the lower portions of the curve to procaterol

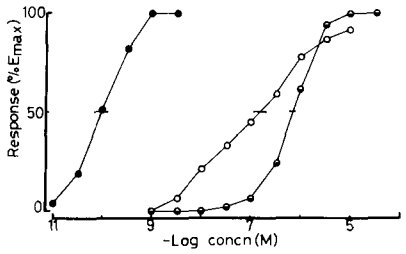

FIG. 1. Mean cumulative concentration-effect curves (n = 4) for the positive inotropic effects of (−)-isoprenaline (●) and procaterol in the absence (○) and presence (◒) of 0·1 μM ICI 118,551 in left atrial preparations from the cat. Each response is expressed as a percentage of the maximum response obtained with (−)-isoprenaline and bars indicate s.e.m. at the EC50 level.

(mean dose–ratio at EC25, 21 ± 6, n = 4) without affecting responses in the upper part of the curve. In other experiments (not shown) 0·1 μM ICI 118,551 produced a 2·9 ± 0·6 (n = 5)-fold parallel shift to the right in inotropic curves to (−)-isoprenaline in cat, but was without effect on (−)-isoprenaline responses in guinea-pig left atria (n = 4).

Concentration-effect curves for the inotropic effects of (−)-noradrenaline, (−)-adrenaline, (−)-isoprenaline and (±)-*N*-*t*-butylnoradrenaline were shifted to the right in a parallel manner by practolol in both guinea-pig and cat left atrial preparations. Table 1 shows mean pK_B values for practolol assessed from these rightward shifts. Whilst the pK_B values for practolol in guinea-pig left atria are independent ($P > 0.05$ Student's paired *t*-test) of the agonist used, those in cat left atria appear to be agonist-dependent. Thus, pK_B values with adrenaline and *N*-*t*-butylnoradrenaline, agonists displaying selectivity for β_2-adrenoceptor mediated responses in-vitro and in-vivo (Dowd et al 1977; Keh et al 1978), were significantly ($P < 0.05$ Student's paired *t*-test) lower than those for noradrenaline (β_1-adrenoceptor selective) and isoprenaline (non-selective).

In cat left atrial membrane preparations, [^{125}I]CYP (10–200 pM) bound in a saturable, reversible manner, without co-operativity to a single high affinity site. The mean dissociation constant (K_D) for [^{125}I]CYP was 22·5 ± 1·7 (n = 3) pM, the Hill coefficient 0·96 ± 0·07 (n = 3) and the maximal density of binding sites 102·3 ± 13·6 (n = 3) fmol mg^{-1} protein. The K_D value for [^{125}I]CYP in cat left atria is similar to that previously determined in guinea-pig left atrial membrane preparations (20·3 pM, McPherson et al 1984). The abilities of the selective β-adrenoceptor antagonists L643,717-01J10 (β_1-selective, Baldwin et al 1983) and ICI 118,551

Table 1. pK_B values for practolol in guinea-pig and cat left atrial preparations with noradrenaline (NOR), adrenaline (ADR), isoprenaline (ISO) and *N-t*-butyl-noradrenaline (NTB) as agonists. In the guinea-pig pK_B values for practolol were independent of the agonist used. In the cat pK_B values were significantly higher for NOR and ISO (*$P < 0.05$ Student's paired *t*-test) than for ADR and NTB.

	NOR	ADR	ISO	NTB
Guinea-pig (n = 4)	7·1 ± 0·1	7·0 ± 0·1	6·8 ± 0·1	6·9 ± 0·1
Cat (n = 6)	6·9 ± 0·2*	6·4 ± 0·1	6·8 ± 0·1*	6·4 ± 0·1

Values shown are mean ± s.e.m. from n experiments.

(β_2-selective, Bilski et al 1983) to displace [^{125}I]CYP bound to cat and guinea-pig left atrial membrane preparations were assessed using 18 concentrations of the drugs ranging from 0·2 nM–0·1 mM for L643,717-01J10 and from 50 pM–20 μM for ICI 118,551. Both antagonists were able to completely displace all specific binding in the two tissue preparations.

Analysis of the displacement data for both antagonists in guinea-pig left atria indicated evidence for only one binding site. The slope factors of the displacement curves were close to unity (Table 2) and analysis of data by LIGAND indicated a significant preference ($P < 0.05$) for a one-site as opposed to a two-site model.

In contrast, the slope factors for the L643,717-01J10 and ICI 118,551 displacement curves in cat left atria were markedly less than unity (Table 2). When the displacement data for L643,717-01J10 was analysed by LIGAND a significant preference for a two-site model was attained ($P < 0.05$). An improved fit of the displacement data for ICI 118,551 was obtained in four experiments, however, statistical significance ($P < 0.05$) was attained in only one.

Table 3 shows the pK_D values for each of the antagonists calculated on the basis of a one-site model in guinea-pig left atria and a two-site model in cat left atria. The pK_D values (Table 3) are closely similar to the respective pK_B values of the antagonists at β_1- and β_2-adrenoceptor sites determined from organ bath studies (Baldwin et al 1983; Bilski et al 1983; McPherson et al 1984). Thus it would appear that the binding sites for each antagonist can be equated with the two subtypes of β-adrenoceptor. The proportions of β_1- and β_2-adrenoceptor subtypes (expressed as a percent total specific binding) delineated by each antagonist are shown in Table 3.

Table 2. Slope factors for displacement of [^{125}I]CYP by L643,717-01J10 and ICI 118,551 in guinea-pig and cat left atrial membrane homogenates.

	L643,717-01J10	ICI 118,551
Guinea-pig	0·96 ± 0·06 (n = 5)	0·99 ± 0·06 (n = 5)
Cat	0·75 ± 0·05 (n = 3)	0·56 ± 0·16 (n = 4)

Values are mean ± s.e.m. from n experiments.

Table 3. Dissociation constants (pK_D) for L643,717-01J10 and ICI 118, 551 and percent of β_1- and β_2-adrenoceptors in guinea-pig and cat left atrial membrane preparations.

	n		pK_D	% Receptor sites
Guinea-pig				
L643,717-01J10	5	β_1	8·82 ± 0·09	100
ICI 118,551	5	β_1	6·77 ± 0·03	100
Cat				
L643,717-01J10	3	β_1	8·79 ± 0·09	77·9 ± 2·9
		β_2	5·57 ± 0·07	22·1 ± 2·9
ICI 118,551	4	β_1	6·89 ± 0·06	86·8 ± 2·1
		β_2	8·83 ± 0·34	13·2 ± 2·1

Values are mean ± s.e.m. from n experiments.

DISCUSSION

In guinea-pig left atrial preparations, the lack of a significant positive inotropic effect with the selective β_2-adrenoceptor agonist procaterol, together with the agonist-independent pK_B values found for practolol, and the data from the radioligand binding studies, strongly suggest that this tissue possesses a homogeneous population of β_1-adrenoceptors. The differences between these findings and those of Johansson & Persson (1983) who showed that the maximal response to procaterol was 29% of that to (−)-isoprenaline, are not marked and probably reflect differences in the strains of guinea-pigs and/or the experimental conditions employed in the two studies.

In a preliminary report, Vlietstra & Blinks (1976) provided evidence which indicated that cat left atrial preparations possessed only β_1-adrenoceptors. However, in a recent study, Kaumann et al (1983) noted that procaterol produced a biphasic concentration-effect curve and on this basis, suggested that the amine interacted with a high and low affinity component in cat left atria. The results with procaterol in the present study confirm and extend those of Kaumann et al (1983). The drug is a full agonist in this tissue and, in addition, it is apparent from the organ bath studies with ICI 118,551, that β_2-adrenoceptor activation is responsible for the major

portion of the inotropic effect of procaterol. In contrast to the results obtained in guinea-pig, the pK_B values calculated for practolol in the cat were dependent on the agonist used. The magnitude of the difference in pK_B values obtained will be dependent on the selectivity of the agonist used and was of the order of 0·5 log units in this study. Despite the small difference, the significantly lower pK_B values obtained when using the relatively selective β_2-adrenoceptor agents adrenaline and *N-t*-butylnoradrenaline, provide supporting evidence for the participation of β_2-adrenoceptors in inotropic responses to drugs in cat left atrial tissue. The age of the cat population used in the present study was generally younger than that of the guinea-pig. This may account for the species variation observed. However, in animals that could be age matched, there still appeared to be qualitative differences in the β-adrenoceptor population of each species.

Previous radioligand binding studies in cat right atrial membrane homogenates indicated that 78 and 22% of the total population of β-adrenoceptors were of the β_1- and β_2-subtype respectively (Hedberg et al 1980). On the basis of the present results, it would appear that cat left atria possess a similar proportion of the two receptor subtypes. It is probable that the small differences in the proportions of the receptor subtypes as determined by each antagonist reflect the relative selectivities of the drugs for β_1- and β_2-adrenoceptors. Thus, while the $\beta_1:\beta_2$-adrenoceptor selectivity for L643,717-01J10 is 145, the $\beta_2:\beta_1$-adrenoceptor selectivity for ICI 118,551 is 49 (McPherson et al 1984). The comparatively smaller degree of selectivity for ICI 118,551 may account for the failure to resolve the displacement curve into a statistically preferred two-site as opposed to a one-site model, even though a two-site model provided a better fit in 3 of 4 experiments.

In conclusion, the results of the present study indicate that the left atria from the guinea-pig possesses a homogeneous population of β_1-adrenoceptors whilst in the cat, a mixture of β_1- and β_2-adrenoceptor subtypes is present.

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REFERENCES

Baldwin, J. J., Denny, G. H., Hirschmann, R. H., Freedman, M. B., Ponticello, G. S., Gross, D. M., Sweet, C. S. (1983) J. Med. Chem. 26: 950–957

Bilski, A. J., Halliday, S. E., Fitzgerald, J. D., Wale, J. L. (1983) J. Cardiovasc. Pharmacol. 5: 430–437

Dowd, H., Keh, G. S., Raper, C. (1977) Br. J. Pharmacol. 60: 197–203

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

Hedberg, A., Minneman, K. P., Molinoff, P. B. (1980) J. Pharmacol. Exp. Ther. 212: 503–508

Johansson, L.-H., Persson, H. (1983) J. Pharm. Pharmacol. 35: 804–807

Kaumann, A. J., Birnbaumer, L., Wittman, R. (1978) in: Birnbaumer, L., O'Malley, B. W. (eds) Receptors and Hormone Action. Academic Press, New York, pp 133–177

Kaumann, A. J., Morris, T. H., Bojar, H. (1983) J. Recept. Res. 3: 61–70

Keh, G. S., Raper. C., Dowd, H. (1978) Clin. Exp. Pharmacol. Physiol. 5: 393–398

McPherson, G. A. (1983a) Comput. Prog. Biomed. 17: 107–114

McPherson, G. A. (1983b) Trends in Pharmacol. Sci. 4: 369–370

McPherson, G. A., Malta, E., Molenaar, P., Raper, C. (1984) Br. J. Pharmacol. 182. 897–904

Munson, P. J., Rodbard, D. (1980) Anal. Biochem. 107: 220–239

Vlietstra, R. E., Blinks, J. R. (1976) Fed. Proc. 35: 210

Zaagsma, J., Oudhof, R., van der Heijden, P. J. C. M., Plantje, J. F. (1979) in: Usdin, E., Kopin, I. J., Barchas, J. (eds) Catecholamines, Basic and Clinical Frontiers. Pergamon Press, Oxford, pp 435–437