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COMMUNICATIONS

Evidence from use of neuronal uptake inhibition that β_1-adrenoceptors, but not β_2-adrenoceptors, are innervated

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It has been suggested by Ariëns that the two β-adrenoceptor subtypes classified as β_1 and β_2 by Lands et al (1967) may alternatively be referred to as β_T and β_H respectively (Ariëns & Simonis 1976; Ariëns 1981). β_T-adrenoceptors were defined as those that respond primarily to neurotransmitter release, while β_H-adrenoceptors were responsive to circulating catecholamines. This concept has received support by Bryan et al (1981), who observed that extraneuronal uptake of catecholamines is active in tissues where β_2-adrenoceptors mediate the sympathetic response but does not appear to be involved where responses are mediated solely via β_1-adrenoceptors; here neuronal uptake was presumed to predominate.

We have determined pharmacologically the role of neuronal uptake in the removal of catecholamines in tissues with either β_1- or β_2-adrenoceptors by examining the effects of the neuronal uptake inhibitor desmethylimipramine (DMI) (Iversen 1965) on the β-adrenoceptor-mediated responses of several tissues. Potentiation by DMI would thus provide an index of sympathetic innervation and enable comparison with the receptor classification into the β_1- or β_2-types.

Materials and methods

Guinea-pigs of either sex (300–500 g) were killed by a blow to the head and exsanguinated. Tissues were rapidly excised and suspended in Krebs-bicarbonate solution (composition (mM): NaCl 118·4, KCl 4·7, $CaCl_2 \cdot 2H_2O$ 1·9, $NaHCO_3$ 25, $MgSO_4 \cdot 7H_2O$ 1·2, glucose 11·7, $KH_2PO_4 \cdot 2H_2O$ 1·2) gassed with 5% CO_2 in oxygen and maintained at 38 °C. All tissues were incubated throughout with phentolamine (5×10^{-6} M) and metanephrine (10^{-5} M) to inhibit α-adrenoceptor stimulation and extraneuronal uptake respectively. All responses were recorded isometrically via a Devices UF1 transducer (57 g sensitivity range) on a Devices M19 polygraph.

Cardiac preparations. Separated left and right atria and left ventricular papillary muscles were dissected away from the ventricular mass and suspended under a resting tension of 0·6–0·8 g. Left atria and papillary muscles were paced at 2 Hz with a threshold voltage (+50%) and 5 ms pulse width using an SRI stimulator (type 6053). Right atrial rate was recorded by means of a ratemeter (Devices, type 2751) triggered by the tension signal. The tissues were allowed to equilibrate for 30 min, changing the bathing medium every 10 min, before drug challenge.

Lung parenchymal strips. Strips of lung parenchyma 1–2 cm long, were prepared as described by Lulich et al (1976) and suspended under a resting tension of 1·5 g. Following 1 h equilibration, with changes of bathing medium every 15 min, the strips were contracted with carbachol ($5{\cdot}8 \times 10^{-6}$ M) prior to recording relaxation by noradrenaline.

Drug administration. Cumulative dose-response curves to noradrenaline were constructed in each tissue before and 30 min after the addition of DMI (10^{-6} M). In cardiac preparations, an initial dose-response curve was constructed but discarded from any calculations, since it has been shown to differ from subsequent dose-response curves (Clark & Poyser 1977). DMI-independent changes in sensitivity occurring between pre- and post-DMI curves were allowed for by performing control experiments in which two noradrenaline dose-response curves were constructed without the intervention of DMI. Mean ($n \geq 4$) responses to each concentration of noradrenaline on the first and second dose-response curves of control experiments were expressed as a fraction. This factor was then applied to the individual corresponding responses on the pre-DMI dose-response curve of test experiments. Responses were then plotted as a percentage of their own maximum tension change and geometric mean ($n \geq 4$) EC50 values were determined and compared statistically by the non-parametric Mann-Whitney U-test (Siegel 1956). Dose-ratios at the EC50 were also determined in individual experiments. The drugs used were desmethylimipramine hydrochloride (Ciba-Geigy), (±)-metanephrine hydrochloride (Sigma), (−)-noradrenaline bitartrate (Sigma) and phentolamine mesylate (Ciba-Geigy). We are grateful to the company for a gift of the latter.

Results

In left atria and papillary muscle, noradrenaline pro-

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Table 1. The effect of desmethylimipramine (DMI, 10^{-6} M) on dose-responses to noradrenaline in guinea-pig isolated tissues. Geometric mean EC50 values for noradrenaline and their 95% confidence limits (in parentheses) were calculated before and in the presence of DMI. The pre-DMI values were corrected from control experiments. The mean (± s.e.m.) ratios of ED50 values were calculated from individual experiments.

Significance levels for differences between EC50 values before and with DMI, as determined by the Mann-Whitney *U*-test are depicted as $^*P < 0.05$, NS not significant.

Tissue	Receptor type	Reference	Response	EC50 (× 10^{-7} M) Before DMI	With DMI	EC50 ratio	n
Left atria	β_1	Zaagsma et al (1979)	Increase tension	7·6 (2·1–27·7)	2·2* (0·8–6·2)	3·6 ± 0·3	5
Papillary muscles	β_1	Zaagsma et al (1979)	Increase tension	12·6 (8·9–17·9)	1·6* (0·4–6·4)	8·9 ± 2·5	4
Right atria	β_1	Zaagsma et al (1979) O'Donnell & Wanstall (1979)	Increase rate	3·3 (1·8–6·1)	0·5* (0·3–1·0)	6·1 ± 0·3	5
Lung strips	β_2	Zaagsma et al (1979)	Relaxation	131·7 (97·7–178·9)	100·2^{NS} (77·7–129·3)	1·3 ± 0·1	5

duced dose-dependent increases in tension with geometric mean EC50 values of 7·6 and 12·6 × 10^{-7} M respectively, while in right atria dose-dependent increases in rate of contraction were observed, with a mean EC50 value of 3·3 × 10^{-7} M. In the presence of DMI (10^{-6} M), noradrenaline dose-response curves in all three tissues were significantly ($P < 0.05$) shifted to the left giving dose-ratios at the 50% response of 3·6 ± 0·3, 8·9 ± 2·5 and 6·1 ± 0·3 respectively (Table 1).

Noradrenaline produced dose-dependent relaxations of the carbachol-contracted lung strip with a mean EC50 value of 131·7 × 10^{-7} M. However, in contrast to the cardiac preparations, DMI had no significant ($P > 0.05$) effect on the dose-response curves, the ratio at the EC50 being 1·3 ± 0·1 (Table 1).

Discussion

From the use of selective β-adrenoceptor agonists and antagonists, the three cardiac preparations used here have been shown to contain a homogeneous population of β_1-adrenoceptors (O'Donnell & Wanstall 1979; Zaagsma et al 1979). In contrast, the lung strip of the guinea-pig contains only β_2-adrenoceptors (Zaagsma et al 1979). DMI shifted the dose-response curves to noradrenaline to the left in the left atria, papillary muscles and right atria. This increase in sensitivity arises as a direct result of neuronal uptake inhibition in these tissues (Matsuo & Toda 1968). In contrast, there was no potentiation of the relaxant response of isolated lung strips. This suggests that only in cardiac preparations is the activity of noradrenaline limited by uptake into neurons adjacent to the β-adrenoceptors, while in the lungs there is no neuronal uptake. We have previously shown that, in contrast to the cardiac preparations, lung strips are insensitive to indirectly acting sympathomimetic amines and to the sensitizing action of chronic reserpine pretreatment (Broadley & Hawthorn 1981). Taken together, these observations support the view that β_1-adrenoceptors receive a nervous innervation whereas β_2-adrenoceptors of the lung appear to be non-innervated and thus support the general concept that β_2-adrenoceptors are responsive primarily to circulating catecholamines (Ariëns 1981).

Examination of this question at vascular β_2-adrenoceptors has yielded contradictory results. By the use of sympathetic nerve stimulation, the β_2-adrenoceptors of the pulmonary vasculature have been claimed to receive a sympathetic innervation (Hyman et al 1981), while those of the dog hind limb vasculature are thought to be non-innervated (Russell & Moran 1980). Furthermore, in contrast to the present findings, Winquist & Bevan (1981) reported a potentiation of the β_2-vasodilator responses of rabbit isolated facial vein by DMI. However, this preparation also contains α-adrenoceptors which are innervated. It is possible therefore that the uptake mechanisms of these nerves could limit the activity of exogenous noradrenaline on neighbouring non-innervated β_2-adrenoceptors. In our study, preparations were carefully selected to have essentially one receptor type. Although α-adrenoceptors may exist in the guinea-pig myocardium, they have a minimal role in the production of inotropic responses and they are not involved in right atrial chronotropic responses (Wagner & Brodde 1978).

In conclusion, the present finding that neuronal uptake removes noradrenaline in tissues with β_1, but not β_2-adrenoceptors, complements the observation of Bryan et al (1981) that extraneuronal uptake does not operate in tissues having only β_1-adrenoceptors.

REFERENCES

Ariëns, E. J. (1981) TIPS 2: 170–172

Ariëns, E. J., Simonis, A. M. (1976) in: Saxena, P. R., Forsyth, R. P. (eds) Beta-Adrenoceptor Blocking Agents. North Holland Publishing Company, Amsterdam, pp 4–27

Broadley, K. J., Hawthorn, M. H. (1981) Br. J. Pharmacol. 73: 202–203P

Bryan, L. J., Cole, J. J., O'Donnell, S. R., Wanstall, J. C. (1981) J. Pharmacol. Exp. Ther. 216: 395–400

Clark, S. J., Poyser, R. H. (1977) J. Pharm. Pharmacol. 29: 630–632

Hyman, A. L., Nandiwanda, P., Knight, D. S., Kadowitz, P. J. (1981) Circ. Res. 48: 407–415
Iversen, L. L. (1965) Adv. Drug Res. 2: 1–46
Lands, A. M., Luduena, F. P., Buzzo, H. J. (1967) Life Sci. 6: 2241–2249
Lulich, K. M., Mitchell, H. W., Sparrow, M. P. (1976) Br. J. Pharmacol. 58: 71–79
Matsuo, S., Toda, N. (1968) Br. J. Pharmacol. Chemother. 32: 473–482
O'Donnell, S. R., Wanstall, J. C. (1979) J. Pharm. Pharmacol. 31: 686–690
Russell, M. P., Moran, N. C. (1980) Circ. Res. 46: 344–352
Siegel, S. (1956) Nonparametric statistics for the behavioural sciences. McGraw-Hill Kogakusha, Tokyo
Wagner, J., Brodde, O.-E. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 302: 239–254
Winquist, R. J., Bevan, J. A. (1981) Circ. Res. 49: 486–492
Zaagsma, J., Oudhof, R., van der Heijden, P. J. C. M., Plantjé, J. F. (1979) in: Usdin, E., Kopin, I. J., Barchas, J. (eds) Catecholamines: Basic and Clinical Frontiers. Pergamon Press, Oxford pp 435–437

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Tolerance and cross-tolerance studies with morphine and ethylketocyclazocine

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It is generally believed that the in vivo effects of opioids are mediated by subclasses of opiate receptors that have been designated as μ, κ and σ (Gilbert & Martin 1976; Martin et al 1976). In this model, agonists are specific for particular receptor subtypes, and the ultimate effects are initiated by an interaction of agonist with receptor subtype. For example, the benzomorphan, ethylketocyclazocine (EK), is thought to cause analgesia in pressure and writhing tests by interacting with κ receptors whereas morphine is active in the same tests through an interaction with μ receptors (Tyers 1980). The view that an effect, in a particular test for analgesia, can be initiated through two distinct receptor subtypes is inconsistent with conventional models of drug action. Traditionally, a pharmacological effect has been associated with a receptor rather than the agonist, with a single effect being mediated through only one receptor subtype. Our previous (Cowan et al 1978) and present studies (involving pressure and heat stimuli, respectively) with EK and morphine suggest that this more traditional view is also applicable to the opioids. Specifically, we believe that morphine and EK, prototype ligands at μ and κ receptors, respectively, cause analgesia in rats through agonist actions on a common receptor.

Methods

Male, Sprague Dawley rats (180–220 g; Zivic-Miller) were implanted s.c. with two pellets each containing 75 mg of morphine alkaloid or with two placebo pellets. The pellets were wrapped in nylon mesh in order to facilitate their removal 72 h after implantation. Dose-response curves for analgesia were obtained with morphine and EK 24 h after pellet removal. The procedure employed was the tail flick test with water at 58 °C as the nociceptive stimulus. Each animal served as its own control. The analgesic effect was calculated using a 15 s cutoff time and the following formula:

% of maximum possible effect = [(test time–control time) × 100]/(15–control time).

Testing took place 30 min after challenging the rats with s.c. morphine sulphate (Mallinckrodt) or ethylketocyclazocine methane sulphonate (Sterling-Winthrop). Doses are given in terms of the salt.

Results and discussion

Doses of morphine necessary to produce analgesia in placebo (control) rats ranged between 2·5 and 20 mg kg^{-1}, s.c. while doses for EK in placebo rats were between 0·2 and 2·5 mg kg^{-1}. In each case, a maximum effect was obtained. In morphine-pelleted animals, the dose necessary to produce analgesia increased to 10–80 mg kg^{-1} for morphine and 10–120 mg kg^{-1} for

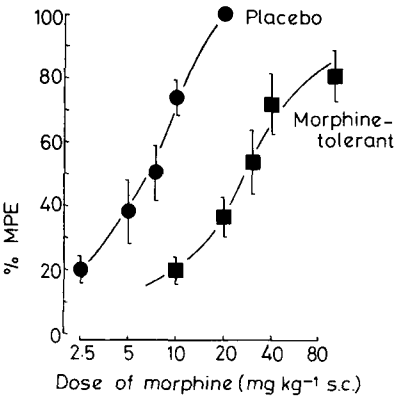

FIG. 1. Percent of maximum possible (analgesic) effect (% MPE) ± s.e. after morphine in either placebo (●) or morphine (■) tolerant rats. Groups of 8–10 animals were used at each dose in the tail flick test.

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