Lack of cross-desensitization between structurally dissimilar α-adrenoceptor agonists

ROBERT R. RUFFOLO, JNR, BETHANY S. TUROWSKI, POPAT N. PATIL*, *Division of Pharmacology, College of Pharmacy, 500 W. 12th Avenue, The Ohio State University, Columbus, Ohio, 43210, U.S.A.*

Recently, we have described the binding of [^{3}H]dihydroazapetine, a reversible α-adrenoceptor antagonist, to a crude membrane fraction from the rat vas deferens (Ruffolo, Miller & others, 1976a; Ruffolo, Fowble & others, 1976b). [^{3}H]Dihydrozapetine binds to a site with many characteristics expected for the α-adrenoceptor. In particular, all α-adrenoreceptor antagonists, and those agonists of the imidazoline class, inhibited specific binding of [^{3}H]dihydroazapetine with excellent correlation between affinity for the binding site and known affinity for the receptor. Direct-acting agonists of the phenethylamine class (*i.e.* structural analogues of noradrenaline), however, paradoxically increased binding. An allosteric effect was postulated to explain these results which are also consistent with the pharmacological experiments of Kalsner (1970, 1973).

The differential effects between agonists of the phenethylamine and imidazoline classes with respect to [^{3}H]dihydroazapetine binding, combined with the marked structural dissimilarities between the two classes, prompted us to propose that imidazolines interact at a different site on the receptor than the phenethylamines. A similar postulation involving different sites of interaction on the α-adrenoceptor for agonists and structurally dissimilar antagonists has been proposed by Ariëns & Simonis (1964). We believe that our pharmacological experiments on receptor desensitization furnish evidence for the existence of different sites of interaction on the α-adrenoceptor for agonists of the imidazoline and phenethylamine classes.

The imidazoline agonists are relatively long-acting and produce receptor desensitization upon repeated administration (Mujic & van Rossum, 1965; Sanders, Miller & Patil, 1975). It was reasoned that if one common binding site, or mode of binding, existed for imidazolines and directly-acting phenethylamines, then desensitization of the α-adrenoceptor to an imidazoline would result in desensitization to other imidazolines as well as the phenethylamines. Conversely, if different sites of interaction were to exist, cross-desensitization between imidazolines and phenethylamines might not occur. It is believed that desensitization experiments may be useful in distinguishing between different receptors (Barsoum & Gaddum, 1935; Waud, 1968; Schild, 1973). The possibility exists, therefore, that the procedure may also discriminate between different sites of interaction on the same receptor protein.

Vasa deferentia were isolated from male albino rats and mounted in constant temperature (37·5°) organ baths for recording isometric drug-induced contractions as described before (Patil, Burkman & others, 1972). Since imidazolines produce periodic spontaneous activity in the vas deferens after the first administration, all subsequent administrations were performed during lulls in the spontaneous activity thereby facilitating assessment of drug-induced contractions. The response of the rat vas deferens to oxymetazoline (an imidazoline, 10 μM) gradually decreases with successive administrations, at 20 min intervals, producing virtually complete desensitization after seven exposures (Fig. 1a). On the desensitized tissue (Fig. 1b), further additions of oxymetazoline fail to produce a significant response while the phenyethylamines, represented by phenylephrine, noradrenaline and methoxamine, produce marked contraction similar to that expected in the normal tissue. The imidazoline, tetrahydrozoline, was unable to elicit a significant response. Before terminating each experiment, 10 μM oxymetazoline was administered to demonstrate that receptors were still desensitized, after which 50 mM KCl was added to rule out the possibility of fatigue as an explanation for the imidazolines inability to elicit a response. Similar results were obtained from tissues isolated from rats pretreated with reserpine (5 mg kg^{-1}, i.p., 17–21 h before death). This treatment

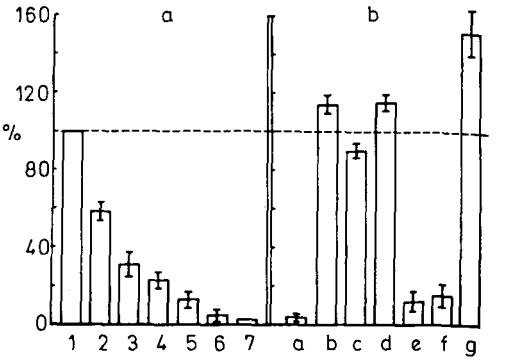

FIG. 1. (a) Contraction of the rat vas deferens (expressed as % of the oxymetazoline maximum) to successive administrations of 10 μM oxymetazoline at 20 min intervals. The number below each column refers to the number of exposures to oxymetazoline. Each column is the mean of 8 observations and the bars are s.e.m.
(b) Ability of various α-adrenoceptor agonists to contract the rat vas deferens after desensitization by oxymetazoline. Each column is the mean of 6 to 8 observations and the bars are s.e.m. a—Oxymetazoline (0·01), b—phenylephrine (0·1), c—noradrenaline (0·01), d—methoxamine (0·1), e—tetrahydrozoline (0·1), f—oxymetazoline (0·01), g—potassium chloride (50) The numbers in parentheses refer to the concentration of agonist used (mM).

* Correspondence.

Table 1. *Effects of various sympathomimetic amines on oxymetazoline-desensitized vas deferens*[a].

Drug	Concn mM	n[b]	Contraction (% of max)[c]
Phenethylamines			
(−)-Phenylephrine	0·1	8	114 ± 5
(−)-Noradrenaline	0·01	6	90 ± 4
(+)-Noradrenaline	3·0	5	61 ± 9
(±)-Methoxamine	0·1	6	115 ± 4
(±)-Cobefrin	1·0	8	82 ± 7
Epinine	3·0	5	90 ± 3
(±)-Synephrine	3·0	5	65 ± 5
(−)-Adrenaline	0·1	5	75 ± 3
(+)-Adrenaline	1·0	5	74 ± 11
(±)-Metaraminol	3·0	5	79 ± 3
(±)-Isoprenaline	100·0	1	81
Dopamine	3·0	8	7 ± 5[d]
Imidazolines			
Oxymetazoline	0·01	7	3 ± 3
Tetrahydrozoline	0·1	8	12 ± 5
Xylometazoline	0·1	4	1 ± 1
Naphazoline	0·1	3	2 ± 2

a Vasa deferentia were desensitized by 6 to 7 administrations of 10 μM oxymetazoline at 20 min intervals with 4 washings between each administration.

b Number of individual observations.

c Data are expressed as % of the oxymetazoline maximum (10 μM).

d Dopamine showed significant cross-desensitization with the imidazolines.

completely abolishes the effects of the indirectly-acting amine, tyramine (Patil, Miller & Trendelenburg, 1974).

The ability of a series of phenethylamine and imidazoline agonists to respond on the oxymetazoline-desensitized vas deferens is shown in Table 1. As is evident, all the phenethylamines tested, with the exception of dopamine, produced significant responses after desensitization whereas the imidazolines do not. The concentration of each drug used was sufficient to produce a response at least 80% of the oxymetazoline maximum.

The cross-desensitization exhibited between the imidazolines and dopamine was quite surprising. It was therefore of interest to study the effects of dopamine on [^{3}H]dihydroazapetine binding to membrane fragments from the rat vas deferens. Whereas noradrenaline, phenylephrine and methoxamine produce stereoselective increases in [^{3}H]dihydrozapetine binding (Ruffolo & others, 1976b) and show a lack of cross-desensitization with the imidazolines, dopamine, which is cross-desensitized by the imidazolines, potently *inhibits* specific binding of the ligand (unpublished observation) as do the imidazolines (Ruffolo & others, 1976b).

The discrepancies between dopamine and the other phenethylamines in binding studies and desensitization experiments suggest that dopamine perhaps acts in a similar manner as the imidazolines which it appears to mimic. The explanation for this observation is unknown at the present time.

Most full agonists produce maximum effects when only a fraction of the total receptor pool is activated. Partial agonists, on the other hand, activate far greater numbers of receptors to produce a maximum response. If desensitization occurs at the level of the receptor, one would expect full agonists to be more resistant to desensitization than partial agonists. Thus, it might be argued that the desensitization observed to the imidazolines is directly related to the fact that these amines, many of which are partial agonists (Mujic & van Rossum, 1965; Sanders & others, 1975), need to activate a greater number of receptors than their phenethylamine counterparts. This explanation seems unlikely since Mujic & van Rossum (1965) have reported that naphazoline and tetrahydrozoline are full agonists on the rat vas deferens, yet both of those compounds are without effect on the oxymetazoline-desensitized vas deferens. Furthermore, the phenethylamine, isoprenaline, is only a very weak α-adrenoceptor agonist and, as such, extremely high concentrations are required (Table 1). It is likely that this weak agonist activates at least as many or possibly more receptors to produce a maximum response than do naphazoline and tetrahydrozoline, yet no signs of cross-desensitization between isoprenaline and the imidazolines were apparent.

The results of the present communication support the contention that imidazoline agonists interact at a different site on the α-adrenoceptor than their phenethylamine counterparts. Easson & Stedman (1933) have presented a conceptual model of the α-adrenoceptor in which the directly-acting phenethylamine agonists interact with complementary sites located in the recognition region of the receptor (Patil & others, 1974). Agonists of the imidazoline class are not close structural analogues of the phenethylamines and would therefore not be expected to fit easily into the Easson-Stedman site. The differences that exist in the effects of the imidazolines and phenethylamines on [^{3}H]dihydroazapetine binding, in addition to the present observations on receptor-desensitization, lead us to suspect that the α-adrenoceptor agonists may act at more than one site on the receptor, the phenethylamines being likely to interact with the classical Easson-Stedman site while the imidazolines interact with another, incompletely understood site. The latter is possibly the same site that we have encountered in biochemical studies with [^{3}H]-dihydroazapetine. Although this site appears to be distinct from the Easson-Stedman site, similar requirements of phenethylamines and imidazolines for a cationic site on the receptor might possibly represent a region of overlap between the two binding sites. If the two classes of agonists bind at different areas on the receptor with perhaps one point of overlap, then it is not surprising that identical pA_2- values for phentolamine

are obtained when phenethylamines and imidazolines are used as agonists (Sanders & others, 1975).

These studies were supported by a grant from the United States Public Health Service, N.I.H. Grant No 9M-17859.

November 4, 1976

REFERENCES

ARIËNS, E. J. & SIMONIS, A. M. (1964). *J. Pharm. Pharmac.*, **16**, 137–157.
BARSOUM, G. S. & GADDUM, J. H. (1935). *J. Physiol. (Lond.)*, **85**, 1–14.
EASSON, L. H. & STEDMAN, E. (1933). *Biochem. J.*, **27**, 1257–1266.
KALSNER, S. (1970). *Life Sci.*, **9**, 961–974.
KALSNER, S. (1973). *Br. J. Pharmac.*, **47**, 386–397.
MUJIC, M. & VAN ROSSUM, J. M. (1965). *Archs int. Pharmacodyn. Thér.*, **155**, 432–448.
PATIL, P. N., BURKMAN, A. M., YAMAUCHI, D. & HETEY, S. (1972). *J. Pharm. Pharmac.*, **25**, 221–228.
PATIL, P. N., MILLER, D. D. & TRENDELENBURG, U. (1974). *Pharmac. Rev.*, **26**, 323–392.
RUFFOLO, R. R., MILLER, D. D., FOWBLE, J. & PATIL, P. N. (1976a). *Pharmacologist*, **18**, 138.
RUFFOLO, R. R., FOWBLE, J. W., MILLER, D. D. & PATIL, P. N. (1976b). *Proc. natn. Acad. Sci., U.S.A.*, **73**, 2730–2734.
SANDERS, J., MILLER, D. D. & PATIL, P. N. (1975). *J. Pharmac. exp. Ther.*, **195**, 362–371.
SCHILD, H. O. (1973). *Drug Receptors*, p. 29. Editor: Rang, H. P. London: MacMillan.
WAUD, D. R. (1968). *Pharmac. Rev.*, **20**, 49–88.

Neuromuscular action of the anticholinesterase RX72601 in the frog

R. WHITTAKER, *School of Pharmacy, Liverpool Polytechnic, Byrom Street, Liverpool, L3 3AF, U.K.*

RX72601 (*cis*-2-(3-hydroxyphenyl)-1-pyrrolidinocyclohexane methobromide) is a member of a series of phenylcyclohexamines which has anticholinesterase properties *in vitro* (Dettmar, Lewis & others, 1974). Metcalf & Dettmar (1975) reported that after injection into anaesthetized animals, RX72601 inhibited acetylcholinesterase in arterial blood and this would explain the antagonism of non-depolarization neuromuscular blockade which they observed in several animal species.

I have used intracellular recording, which is a precise technique for evaluating drug action on neuromuscular transmission (Riker & Okamoto, 1969) to provide information on the mode of action of the drug and to ascertain if a pre-junctional action increasing transmitter release is also present, which would account in part for its anti-curare property.

Experiments were made on frog (*Rana temporaria*) isolated sartorius nerve muscle preparations at 17–20° employing the usual techniques for intracellular recording (Fatt & Katz, 1951) with glass capillary microelectrodes filled with 3 M KCl and resistances 5–10 Mohms, connected through a cathode follower to a Tektronix Type 502A dual beam oscilloscope. Simultaneous d.c. recording was made on paper using an ink-writer Mingograf 34, at low amplification for membrane potentials and a.c. recording at higher amplification for miniature endplate potentials (m.e.p.p.s.) and endplate potentials (e.p.p.s.). The frequency response of the recording system was flat from 5–500 Hz (−3db at Hz and 700 Hz). The composition of the standard hysiological solution was (mM): NaCl, 103; KCl, 1; aCl_2, 1·8; $NaHCO_3$, 1·2.

The electrode was inserted at an endplate and when the membrane potential was stable m.e.p.p.s. were recorded as controls. With the electrode still in position the effect of RX72601 on m.e.p.p.s. was tested either by adding a solution of the drug to the bath fluid or exchanging the bath fluid for one containing the drug and recording m.e.p.p.s. for periods up to 5 min. The bath fluid was then replaced by normal Ringer solution and further records taken. To avoid cumulative effects, in each experiment the effect of one drug concentration only was recorded from a single muscle fibre. Concentrations of RX72601 (2×10^{-8}M and over) increased m.e.p.p. amplitude without altering discharge frequency or the resting membrane potential (Fig. 1a and b). From 4 experiments the mean % increase in amplitude of m.e.p.p.s. (with s.d.) induced by RX72601 (2×10^{-8}M) was 90(18). At high concentrations of 1×10^{-6}M membrane depolarization was not > 5 mV.

To observe the effect of the drug on e.p.p.s., neuromuscular transmission was blocked by adding a solution of tubocurarine chloride to the bath fluid and e.p.p.s. were elicited in response to nerve stimulation at 1 Hz. A solution of RX72601 was then added to the bath fluid and further records from the same endplate showed increased amplitude of e.p.p.s. (Fig. 1c and d). 12 experiments were undertaken to provide an estimate of the mean acetylcholine quantum content of e.p.p.s., by analysis of their amplitude variance using a single muscle fibre recording in each experiment. The formula used to determine mean acetylcholine quantum content (m) of e.p.p.s. was: $m = (\text{mean e.p.p.})^2/(\text{e.p.p. variance})$. At least 100 e.p.p.s. were recorded as controls and in the