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Mouse caecum as a selective β-adrenoceptor tissue

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The mouse isolated caecum has been found to contain β-adrenoceptors and 5-hydroxytryptamine receptors. Conditions for optimum function of the tissue in an isolated environment are described. The preparation is sturdy, lasting, economic and dependable for the screening of potential drugs, possessing agonistic or antagonistic activity on the extra-cardiac β-adrenoceptors.

Barsoum & Gaddum (1935) described the use of fowl caecum as an isolated tissue sensitive to the relaxant action of adrenaline which was subsequently shown to be blocked by dichloroisoprenaline (Cleugh & Gaddum, 1961), suggesting that the fowl caecum contains β-adrenoceptors. As the caecum of small rodents may also contain the β-adrenoceptors, we have examined the caecum of the mouse with the object of characterizing the receptors.

MATERIALS AND METHODS

Agonists and antagonists

(±)-Isoprenaline hydrochloride and (−)-phenylephrine hydrochloride, Winthrop, New York; and (−)-adrenaline and (−)-noradrenaline both as bitartrate salts, U.S.P. Reference Standards as well as Nutritional Biochemicals, Ohio, were used as agonists. (±)-Propranolol hydrochloride and practolol, Ayerst, New York; azapetine phosphate, Hoffman-LaRoche, New Jersey; and (−)-ergotamine tartrate, Sandoz, New Jersey, were used as the adrenoceptor blockers. Other agents used as agonists to characterize the receptors were 5-hydroxytryptamine creatinine sulphate (5-HT), Sigma Chemicals, Missouri; dopamine hydrochloride and acetylcholine chloride, Nutritional Biochemicals, Ohio; and histamine acid phosphate, Lilly, Indiana. Chlorpromazine hydrochloride, Smith, Kline and French, Pennsylvania was used as a blocker for the 5-HT response of the caecum.

The caecum preparation

Laboratory-bred Swiss mice of either sex, 22–28 g, were killed by a blow on the head and by carotid bleeding. The caecum was removed at the ileoceacal junction, transferred to Tyrode solution (containing half strength Ca^{2+} of the following composition (mM): KCl 2·6, $CaCl_2$ 0·9, $MgCl_2$ 1·1, Na_2HPO_4 0·35, $NaHCO_3$ 11·9, NaCl 136·8, dextrose 5·6; pH = 7·4 ± 0·2), and the contents were flushed out with tyrode solution from a pipette. The tissue, threaded at both ends, was mounted at a tension of 0·5 g in a 10 ml bath containing Tyrode solution at 32° (to reduce spontaneous activity) gassed with 5% carbon dioxide in oxygen. The tissue was allowed to

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equilibrate for 1–2 h during which it was washed every 10–15 min. The preparation was responsive for 6–12 h. Caeca from females exhibited less spontaneous activity than from males.

Testing procedures for caecum

The agonists were allowed to equilibrate with the tissue for 2 min or until a maximum response, whichever occurred first; the blocking drugs were added 2–3 min before the agonists. Before testing another dose of blocking drugs, the recovery of the tissue from the effect of the previous dose was ensured by intermittant checking of the response to a standard dose of the agonist. Cumulative dose-response curves were obtained according to Van Rossum (1963).

Other tissues

The relative activities of (—)-noradrenaline and (—)-adrenaline on other preparations have also been obtained for comparison with their activity on the caecum.

Guinea-pig tracheal chain. This preparation was set up according to Long & Chiou (1970). The chain was tensed with 1–20 μg/ml of acetylcholine each time before determining the response to the catecholamines (Kaul, 1971). Usually 15 min were allowed between the doses of the agonists during which the tissue was washed frequently.

Mouse uterus. This tissue was isolated according to Long & Chiou (1970) and Diamond & Brody (1966) and mounted in DeJalon solution at 28° bubbled with 5% carbon dioxide in oxygen. The comparative inhibitions of the tissue response to ED50 of acetylcholine produced by various doses of (—)-adrenaline and (—)-noradrenaline were determined to obtain the relative β-adrenoceptor activities of the two amines.

RESULTS AND DISCUSSION

Response to adrenoceptor agonists

The response of caecum to equivalent concentrations (w/v) of various agonists is shown in Fig. 1A. Isoprenaline effectively relaxed the tissue whereas adrenaline and noradrenaline showed relatively less activity. Phenylephrine, an α-stimulant, even in exponential amounts (Fig. 1B) was virtually devoid of any activity, demonstrating the

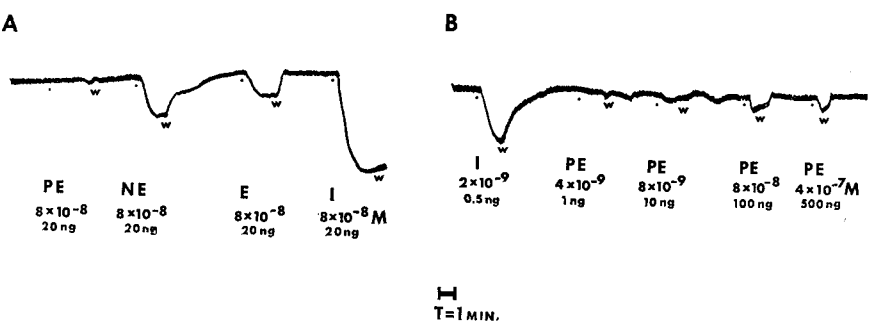

FIG. 1. Relaxant action of various adrenergic agonists on the isolated mouse caecum. Dots, where the drugs were added; w, when the tissue was washed, PE, phenylephrine; NE, noradrenaline; E, adrenaline; I, isoprenaline.

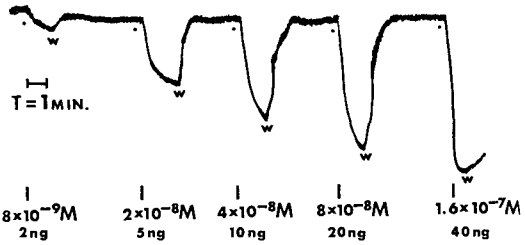

FIG. 2. Dose-response relation of (−)-isoprenaline on the mouse isolated caecum—tracing of the record of the relaxation produced by varying doses of the β-stimulant.

relative absence of α-adrenoceptors in the mouse caecum. Fig. 2 shows a dose-dependent response of the caecum to isoprenaline which is virtually linear in the dose range tested.

Contrary to the general observation when noradrenaline and adrenaline are tested on conventional β-adrenoceptor tissues such as the uterus, trachea and heart, noradrenaline consistently produced a greater response than adrenaline (Fig. 1A). To investigate further, U.S.P. Biological Reference Standards of the two amines and other standard samples (Nutritional Biochemicals Company, Ohio) were tested in equimolar concentrations on mouse uterus and the guinea-pig tracheal chain. On the uterus, the relative blocking activities of the amines were measured against a fixed dose of acetylcholine, whereas on the acetylcholine-tensed tracheal chains the relative degree of relaxation was determined. Table 1 includes the values of relative responses to various equimolar concentrations of adrenaline and noradrenaline on these two tissues compared with the responses of caecum. The data clearly show that on the mouse caecum noradrenaline has a stronger β-stimulant activity than adrenaline. A similar observation on rabbit duodenum has been made by Furchgott (1960), but this tissue has both α- and β-adrenoceptors. Further, these experiments were made in the presence of both the α- and the β-adrenoceptor blocking drugs which antagonize each other's activity (Krell & Patil, 1969; Vaishnav, Pandya & others, 1971).

That the response of mouse caecum to noradrenaline has no α-component is evident from Fig. 3 which shows that the α-adrenoceptor blockers, azapetine and ergotamine,

Table 1. *Relative responses of different β-adrenoceptor tissues to varying equimolar doses of adrenaline and noradrenaline.*

Tissue	Dose (M)	Relative response* Adrenaline	Noradrenaline
Uterus (Mouse)	3×10^{-8}	416	100
	5×10^{-8}	230	100
Tracheal chain (Guinea-pig) ..	6×10^{-6}	600	100
	8×10^{-6}	280	100
	1×10^{-5}	210	100
Caecum (Mouse)	3×10^{-8}	59	100
	6×10^{-8}	66	100
	9×10^{-8}	55	100
	$1{\cdot}6 \times 10^{-7}$	50	100

* Responses are represented relative to noradrenaline response taken as 100. Each relative response value against a particular dose is an average of 2 to 3 observations made on individual tissue preparations.

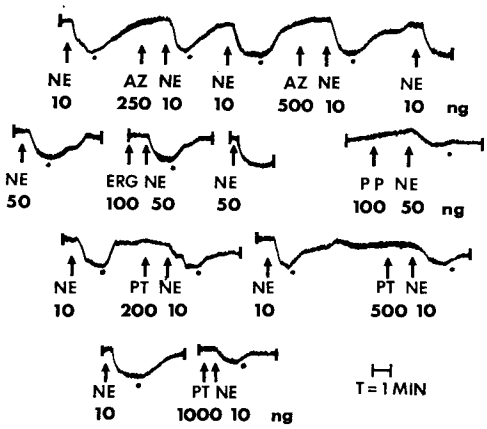

FIG. 3. Blockade of the response of caecum to noradrenaline by various blockers. NE, noradrenaline; Az, azapetine; ERG, ergotamine; PP, propranolol; PT, practolol. Arrows, where drugs were added; dots, when tissue was washed.

did not block the response whereas β-blockers propranolol and practolol, effectively, but not completely, blocked the response to noradrenaline. Attempts to obtain a total block of this response with high concentrations of β-blockers were unsuccessful.

Confirmation by antagonists

Propranolol on known β-adrenoceptor tissues is believed to be a competitive antagonist for isoprenaline, and in low concentrations it occupies the β-adrenoceptors selectively. Fig. 4 represents registrogram tracings showing that the response of the caecum to isoprenaline was blocked by propranolol and practolol. Cumulative dose-response curves of isoprenaline in the presence of varying concentrations of the antagonist, propranolol, are shown in Fig. 5. A parallel shift of the curves to the

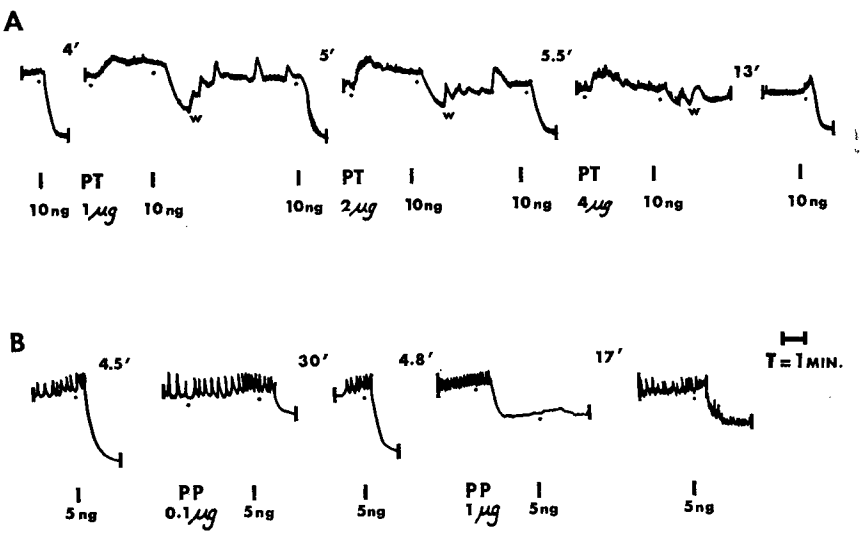

FIG. 4. Blockade of isoprenaline activity by specific β-blockers. I, isoprenaline, PP, propranolol; PT, practolol.

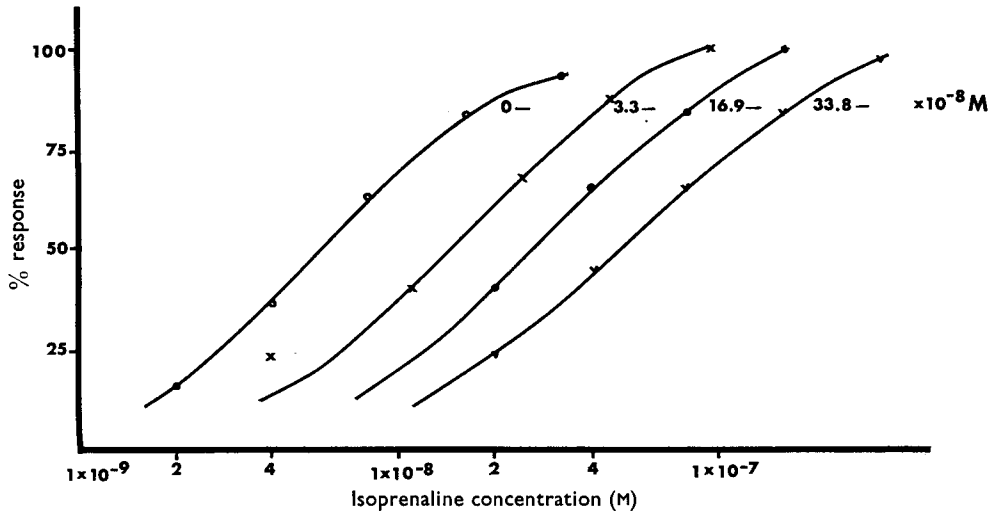

FIG. 5. Cumulative log dose-response curves of isoprenaline activity on the mouse isolated caecum in the presence of varying concentrations of propranolol (as shown in curves).

right on log-dose axis occurred as the concentration of the blocker in the bath was increased. This is typical of a competitive antagonism (Van Rossum, 1963).

Taken together, the data presented clearly suggests that the mouse caecum contains relatively pure β-adrenoceptors.

Other receptors

Since the nature of the receptors of mouse caecum has not been previously reported, we investigated the presence or absence of receptors sensitive to other autacoids. Fig. 6 shows that the mouse caecum contracted in response to 5-HT, indicating that it also possesses 5-HT receptors. The response was blocked by chlorpromazine (Fig. 6B) which competitively blocks the action of 5-HT on other 5-HT receptor tissues

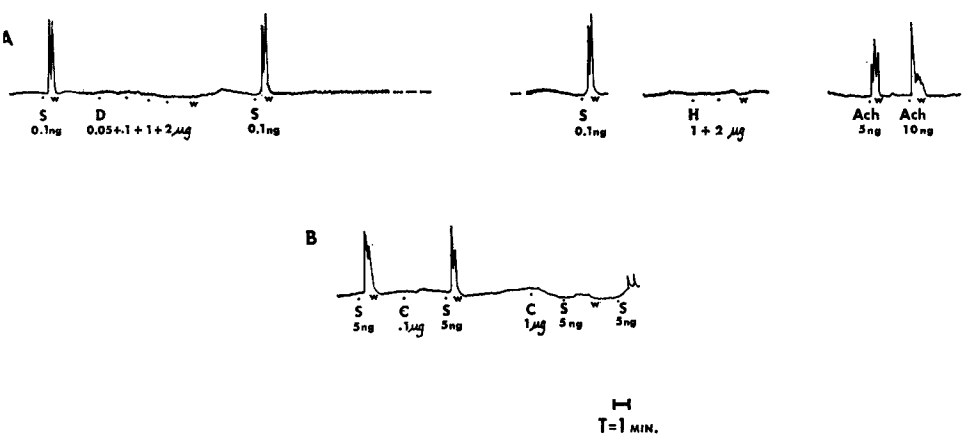

FIG. 6. Response of the mouse isolated caecum to various autacoids (A), and the blockade of 5-HT receptors by chlorpromazine (B). S, serotonin (5-HT); D, dopamine; H, histamine; ACh, acetylcholine; C, chlorpromazine. Dots, where drugs were added; w, when tissues washed.

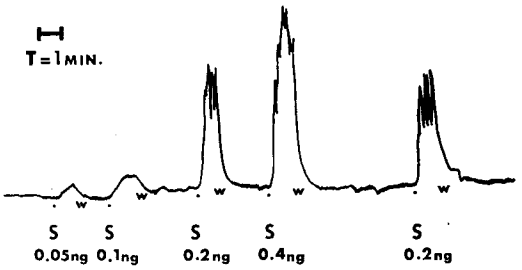

FIG. 7. Dose-dependent spasmogenic response of the mouse caecum to 5-HT.

(Gyermek, 1966). Fig. 7 demonstrates that the mouse caecum exhibited a dose-dependent response to 5-HT, and that this relation was linear on a semi-logarithmic plot in the 15 to 75% range of response.

Acetylcholine produced an expected spasmogenic response in the caecum, but to a far less extent than the respective pharmacological effects of either 5-HT or isoprenaline. Histamine, on the other hand, induced no activity even in a dose thirty thousand times that of 5-HT (Fig. 6). Since not all of the response to noradrenaline could be blocked by a β-blocker, some other yet unknown type of dopamine receptors might be present in the mouse caecum. However, in doses twenty thousand times those of 5-HT, dopamine induced no response in the tissue (Fig. 6).

It is not surprising that the caecum was nearly insensitive to histamine, for rodents are resistant to it (Douglas, 1970). High sensitivity of the tissue to 5-HT supports that this amine may be involved in hypersensitivity reactions in rodents.

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