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Adenyl cyclase activity of rabbit aorta

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Adenyl cyclase activity in homogenates of thoracic and abdominal aorta from young and old rabbits was neither stimulated nor inhibited by isoprenaline, noradrenaline, 5-hydroxytryptamine, or histamine but was markedly increased by sodium fluoride. Sodium fluoride-stimulated adenyl cyclase activity was significantly higher in the abdominal than in the thoracic aorta. Neither the basal nor sodium fluoride-stimulated adenyl cyclase activity of thoracic aorta changed with increasing age of the aorta. On the other hand, the abdominal aorta showed an increase in sodium fluoride-stimulated activity but no change in basal activity with increasing age. After the ATP pool of intact thoracic aorta was prelabelled with [^{14}C]-adenine, isoprenaline did not enhance the formation of labelled cyclic AMP in intact aortic strips. Based on these data no correlation could be made between adenyl cyclase activity and the β-receptor activity of this tissue.

Fleisch, Maling & Brodie (1970) showed that rabbit thoracic but not abdominal aorta could be relaxed by isoprenaline, a β-receptor agonist. In addition this activity of isoprenaline on thoracic aorta decreased with increasing age of the aorta. Since Robison, Butcher & Sutherland (1967) have postulated that adenyl cyclase (AC) is part of the β-adrenergic receptor system in many tissues, we examined AC activity of thoracic and abdominal aorta of young and old rabbits to determine if there was a relation between AC activity and the reported responses to isoprenaline in aortic smooth muscle. The data obtained did not permit a correlation to be made.

MATERIALS AND METHODS

Materials were obtained from the following sources: ATP-^{3}H(G) (15·7 Ci/mmol), New England Nuclear Corp.; [8–^{14}C]adenine (50 mCi/mmol), Schwarz Bioresearch; theophylline, Z. D. Gilman Inc.; (−)-isoprenaline (+)-bitartrate and (−)-noradrenaline-bitartrate, Winthrop laboratories; 5-hydroxytryptamine creatinine sulphate (5-HT), Aldrich Chemical Co.; histamine dihydrochloride, Mann Research Laboratories; dibenamine HCl, gift of Smith, Kline and French; phentolamine methanesulphonate, gift of Ciba; and propranolol HCl, gift of Ayerst Laboratories.

Male and female New Zealand rabbits (1·6–3·3 kg) of known age were used. The animals were killed by administration of 20 to 30 ml of air into the marginal ear vein. Spirally cut thoracic and abdominal aortic strips were prepared by the method of Furchgott & Bhadrakom (1953). The tissues were kept moist with Krebs-bicarbonate solution during the preparation. The aortas were then homogenized in an all-glass

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homogenizer for about 1 min with 10 volumes of a buffer containing 80 mM Tris-HCl pH 7·4, 6·6 mM $MgSO_4$ and 2 mM theophylline.

For each experiment aortic tissues of either one old or two young rabbits were used. Incubations were performed at 30° for 10 min in a total volume of 0·6 ml containing homogenate equivalent to 40 mg of tissue, 2 mM ATP-^{3}H (specific activity 100–200 μCi/μmol), and either 10^{-3}M drugs or 10^{-2}M sodium fluoride. The AC activity was measured by the method of Krishna, Weiss & Brodie (1968). The protein content of homogenates was determined by the method of Lowry, Roseborough & others (1951).

To measure adenyl cyclase activity of intact tissue, a modification of the method of Shimizu, Daly & Creveling (1969) was used. Aortic strips were pre-incubated with [^{14}C] adenine for 45 min in Krebs-bicarbonate solution at 37°. The tissues were then washed and incubated for 1, 2 and 10 min with isoprenaline in the presence of phentolamine (3 μg/ml). The cyclic AMP formed was measured by the method of Krishna & others (1968).

RESULTS AND DISCUSSION

Table 1 shows the AC activity of thoracic and abdominal aortas from young (8 weeks old) male and female rabbits. Since there were no sex differences in the AC activities, data from both sexes were pooled. Although the basal AC activity appeared to be higher in abdominal aorta, the difference was not statistically significant. At concentrations of 10^{-3}M, isoprenaline, 5-HT and histamine did not change the AC activity in thoracic or abdominal aorta. NaF (10^{-2}M), however, significantly increased the AC activity in both thoracic and abdominal aorta. After NaF stimulation the AC activity of abdominal aorta was significantly higher than that of thoracic aorta (Table 1).

Table 2 shows the AC activity of thoracic and abdominal aortas from 2- to 3-year-old rabbits. As with young animals, there was no stimulation by isoprenaline, 5-HT, or histamine, whereas NaF significantly increased the AC activity of both thoracic and abdominal tissue. In contrast to the results obtained in tissue from young animals, however, the basal as well as the NaF-stimulated AC activity was significantly higher in abdominal than in thoracic aorta.

Table 1. *Adenyl cyclase activity in homogenates of aortic tissue from* 8*-week-old rabbits*

	Thoracic aorta		Abdominal aorta	
Drugs	No. Exp.	pmol cAMP/mg protein/min	No. Exp.	pmol cAMP/mg protein/min
No drug	10	1·79 ± 0·29	10	2·90 ± 0·61
		$P < 0·01$		$P < 0·01$
10^{-2}M NaF	10	3·91 ± 0·53	10	6·27 ± 0·54
10^{-3}M Isoprenaline	6	1·83 ± 0·52	5	3·31 ± 0·87
10^{-3}M 5-HT	3	1·20 ± 0·31	3	1·72 ± 0·82
10^{-3}M Histamine	3	1·31 ± 0·34	3	2·19 ± 0·84
mg protein/g tissue	10	124 ± 7	10	97 ± 4

Values are means ± s.e. of the number of experiments indicated. Significant differences between groups were established by paired comparison (Student's *t*-test).
cAMP = cyclic AMP.

Table 2. *Adenyl cyclase activity in homogenates of aortic tissues from* 2- *to* 3-*year-old rabbits*

Drugs	Thoracic aorta No. Exp.	Thoracic aorta pmol cAMP/mg protein/min	Abdominal aorta No. Exp.	Abdominal aorta pmol cAMP/mg protein/min
No drug	4	1·51 ± 0·19	4	3·51 ± 0·35
		$P < 0·01$		$P < 0·01$
10^{-2}M NaF	4	4·68 ± 0·28	4	9·48 ± 0·96
10^{-3}M Isoprenaline	3	1·54 ± 0·22	3	3·95 ± 0·53
10^{-3}M 5-HT	4	1·25 ± 0·16	4	3·30 ± 0·37
10^{-3}M Histamine	4	1·40 ± 0·20	4	4·06 ± 0·58
mg protein/g tissue	4	116 ± 7	4	90 ± 18

Values are means ± s.e. of the number of experiments indicated. Significant differences between groups were established by paired comparison (Student's *t*-test).

A comparison of Tables 1 and 2 shows no marked differences in basal AC activities between young and old rabbits. After NaF stimulation, however, aortas from older animals showed a higher enzymatic activity in abdominal (young, 6·27 ± 0·54; old, 9·48 ± 0·96), but not in thoracic aorta (young, 3·91 ± 0·53; old, 4·68 ± 0·28). These age differences could not be explained by a change in the tissue protein content used as basis for the calculation of AC activity, since the protein content did not change with age. There was, however, a lower protein content in abdominal than in thoracic aorta (Tables 1 and 2).

Turtle & Kipnis (1967) and Abe, Robison & others (1969) showed that α-receptors are capable of mediating a fall in the level of cyclic AMP. Since isoprenaline can act as an agonist at α-receptors, experiments were made in which thoracic aortas from young rabbits were preincubated with an α-receptor blocking agent (1 μg/ml of either dibenamine or phentolamine) for 15 min before homogenization. No change in either basal AC activity or the activity in the presence of isoprenaline was observed. In another experiment, 10^{-3}M noradrenaline was used as an α-receptor agonist. Again, there was no change in the AC activity.

It also seemed possible that homogenization stimulated AC activity in some unknown way such that β-receptor activation could not manifest itself or that homogenization altered the mechanism responsible for β-receptor activation of AC. To overcome these possible objections, thoracic and abdominal aortas from young animals were treated with 10^{-3}M isoprenaline for 1 min after 3 μg/ml phentolamine. The aortas were then homogenized in the presence of 10^{-3}M isoprenaline and AC activity was measured in the presence of 10^{-3}M isoprenaline as described above. Neither isoprenaline nor phentolamine changed the basal AC activity (Table 3). Thus, under these conditions AC activity cannot be changed by β-receptor activation even when the tissue is intact. To preclude the possibility that adenyl cyclase was maximally stimulated by a substance released during homogenization, aortas were preincubated with 10^{-6}M propranolol, a β-receptor blocking agent, for 15 min, then homogenized in 10^{-6}M propranolol and the AC assayed. Propranolol did not influence the basal activity, indicating that homogenization plays no role in the stimulation of AC through a β-receptor mechanism (Table 3). In both sets of experiments, 10^{-2}M NaF added after homogenization, markedly increased AC activity (Table 3).

Table 3. *Adenyl cyclase activity in homogenates of rabbit aorta during adrenergic blockade**

Drugs added before homogenization	Drugs added during assay	Thoracic aorta pmol cAMP/mg	Abdominal aorta protein/min
None	None	1·63	3·00
3 μg/ml Phentolamine	3 μg/ml Phentolamine	1·54	2·53
3 μg/ml Phentolamine	NaF	4·18	8·35
3 μg/ml Phentolamine and 10^{-3}M Isoprenaline	10^{-3}M Isoprenaline	1·60	2·68
10^{-6}M Propranolol	10^{-6}M Propranolol	1·62	3·21

* Pool of three rabbit aortas. Each value is the result of duplicate determinations.

Isoprenaline is known to enhance activity more effectively in intact heart (Laraia & Reddy, 1969), fat cells (Kuo & Renzo, 1969) and brain slices (Shimizu & others, 1969) than in homogenates. For this reason, adenyl cyclase activity was measured in intact aortas prelabelled with [^{14}C]adenine for 45 min at which time, 85 to 90% of the total radioactivity in the tissue can be found in the ATP fraction (Krishna & others, 1968). In the presence of 5×10^{-4}M theophylline and 3 μg/ml of phentolamine isoprenaline 10^{-3} and 10^{-6}M failed to induce significant conversion of ATP into cyclic AMP at 1,2 and 10 min (pool of 6 thoracic aortas).

The present study shows that the AC activity in aortic tissue cannot be stimulated by various biogenic amines but is increased by 10^{-2}M NaF after homogenization. It also indicates that, although there are no age differences in the endogeneous AC activity of either the thoracic or abdominal aortic strips, the NaF-stimulated activity of the abdominal aorta increased with age. Thus, the difference in responsiveness to a β-adrenergic receptor agonist between thoracic and abdominal aorta and the decrease of this responsiveness with increasing age found by Fleisch & others (1970) could not be related to changes in AC activities of these tissues. It is still possible, however, that these negative results are due to the heterogeneity of aortic tissue and to the fact that β-receptors represent a small part of the whole tissue.

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