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Dopamine-sensitive adenylate cyclase in canine renal artery

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To characterize further a putative dopamine receptor in the renal artery, the effects of dopamine on canine renal artery adenylate cyclase activity were studied. Since the femoral artery is thought to be devoid of a similar dopamine receptor, the effects of dopamine on the adenylate cyclase activity of the canine femoral artery were also studied. In tissues from dogs with or without phenoxybenzamine pretreatment, renal artery adenylate cyclase was maximally stimulated by 4 μM dopamine, compared to 20 μM required for the femoral artery enzyme. The concentrations of isoprenaline required to maximally stimulate renal and femoral artery adenylate cyclase were 0·04 and 0·2 μM, respectively. In tissue from the phenoxybenzamine-pretreated dog, the stimulatory effect of dopamine on the renal artery enzyme was selectively blocked by 0·01 μM haloperidol, but not by 0·2 μM propranolol. In the femoral artery, however, the dopamine stimulation was blocked by both antagonists. Stimulation by isoprenaline of renal and femoral artery adenylate cyclase was blocked by propranolol. These data suggest the concept that dopamine interacts with a specific artery receptor apparently different from α- and β-adrenoceptors.

Dopamine-induced vasodilation has been demonstrated in the canine renal vascular bed but not in the femoral vascular bed (for a review, see Goldberg, 1972). Changes in cyclic AMP have been proposed to be associated with vascular relaxation (Triner, Vulliemoz & others, 1972). To characterize the putative receptor subserving the specific action of dopamine, the effects of the amine on adenylate cyclase activity in renal and femoral arteries of the dog were compared.

The β-adrenoceptor agonist, isoprenaline, has been shown to stimulate vascular adenylate cyclase (Triner, Nahas & others, 1971). Therefore we have compared the effects of this agent and dopamine. In addition, limited studies on the effects of these two compounds after pretreatment with the α-adrenoceptor blocking agent, phenoxybenzamine, were conducted either in the presence or absence of the β-adrenoceptor blocking agent, propranolol as well as the putative dopamine receptor blocking agent, haloperidol (Yeh, McNay & Goldberg, 1969)†.

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MATERIALS AND METHODS

Materials

Dopamine and theophylline were obtained from Calbiochem; (±)-propranolol (Inderal) from Ayerst Laboratories; haloperidol (Haldol) from McNeil Laboratories; (±)-isoprenaline, creatine phosphate and creatine phosphokinase, from Sigma Chemical Company. AG 50W-X8, 100–200 mesh (H^+ form) was obtained from BioRad. All other chemicals were of reagent grade and were purchased from standard commercial sources. ATP-2,8-[^{3}H], tetrasodium salt (New England Nuclear) was supplied as a solution in 50% ethanol. Before use, ethanol was removed by evaporation; a final concentration of 2·5 mM ATP was made by adding non-radioactive ATP (Boehringer-Mannheim). The purity of radioactive ATP was >99·8%.

Methods

Female mongrel dogs (14–20 kg) were maintained on a Purina Dog Chow and were allowed free access to water. All animals were anesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). Phenoxybenzamine (10 mg kg^{-1}) was administered by intravenous infusion to some of the animals before dissection of the femoral and renal arteries. The arteries were immediately transferred to ice cold 0·9% NaCl solution.

The tissues were exposed by a retroperitoneal flank incision. The main renal artery was dissected free from its origin at the aorta to its first bifurcation.

The outside diameter of renal arteries ranged from 1·5 to 5 mm. The femoral artery was dissected from its origin in the femoral triangle to the origin of the saphenous artery. The outside diameter of femoral arteries ranged from 3 to 4 mm.

Preparation of tissues. Two different tissue preparations were used. (1) *Homogenates.* Homogenates were prepared by gentle homogenization of arteries (ranging from 0·5–2·5 g) in a Potter-Elevejhem homogenizer fitted with a motor driven Teflon pestle, using 9 volumes of 50 mM tris-Cl buffer, pH 7·5. (2) *Particulate fraction.* Arteries were subjected to homogenization for 10 s in 3 ml of 0·32 M sucrose in the above tris-Cl buffer using a Polytron homogenizer (Brinkmann) and transferred to a centrifuge tube. The tissue remaining in the homogenizer was rehomogenized with an equal volume of sucrose buffer-solution. The pooled homogenate was centrifuged at 5000*g* for 10 min. The resulting pellet was suspended in a total volume of 3–5 ml of 0·25 M sucrose-buffer solution, to yield the particulate fraction.

Assays. Adenylate cyclase was measured by a modification of the procedure of Krishna, Weiss & Brodie (1968). A third $BaSO_4$ precipitation step was included to ensure complete removal of unreacted ATP remaining at the end of incubation. Reactions were carried out in 1·2 × 10 cm tubes for 15 min at 30°, with shaking. Each tube contained in a final volume of 0·25 ml, 50 mM tris-Cl buffer, pH 7·5; 5 mM $MgSO_4$; 2 mM cyclic AMP; ATP regenerating system consisting of 24 mM creatine PO_4 and 300 μg creatine phosphokinase; 5 mM theophylline; 0·1 mM [^{3}H]-ATP containing about 1×10^6 counts min^{-1}; and 0·1 ml of tissue fraction equivalent to about 200 μg protein. Reaction was initiated by adding the substrate and the incubation was terminated by placing the tubes in a boiling water bath for 2 min. Water was added to bring the volume in each tube to 1 ml. After centrifugation at 1500*g* for 3 min, the supernatant was assayed for cyclic AMP formed. Protein concentration in tissue fractions were determined by the procedure of Lowry, Rosebrough & others (1951). A unit of activity of the enzyme is defined as p mol of cyclic AMP formed mg^{-1} protein min^{-1}.

Statistics. The significance of the difference between two mean values were assessed by the Student's *t*-test (Steele & Torrie, 1960).

Table 1. *Adenylate cyclase activity in homogenates of renal and femoral arteries of dog.* Homogenates were incubated for 15 min at 30°, with or without addition, in the presence of 5 mM theophylline. Adenylate cyclase activity is expressed as % of basal activities, which represent 3·60 ± 0·47 and 3·73 ± 1·00 units, for renal and femoral arteries respectively. Each value presented is mean ± s.e.m. of five separate experiments. Each experiment was performed in triplicate incubations.

	Adenylate cyclase activity (% basal)	
Addition	Renal	Femoral
None (basal)	100 ± 31	100 ± 26
Dopamine, 4 μM	413 ± 45*	—
Dopamine, 20 μM	—	214 ± 21*
(±)-Isoprenaline, 0·04 μM	330 ± 16*,†	—
(±)-Isoprenaline, 0·2 μM	—	347 ± 41*
NaF, 10 mM	250 ± 33*	311 ± 28*

* Significantly different from basal activity ($P < 0.05$).

† In one experiment the results using 0·01, 0·02, 0·04, 0·16, 0·2 and 0·4 μM isoprenaline respectively, were 192, 293, 370, 300, 140 and 135% of basal activity of renal artery.

RESULTS

Adenylate cyclase activities in renal and femoral arteries of the dog

Homogenates. Adenylate cyclase activity in the homogenates of renal artery was stimulated by both isoprenaline and dopamine. The stimulation was maximal with 0·04 μM isoprenaline (Table 1), whereas dopamine-induced stimulation did not appear to be concentration dependent within a range of concentrations of 3 to 10 μM (Fig. 1). The enhancement of adenylate cyclase activity by dopamine was about twice that produced by 10 mM NaF (Table 1). The concentrations of isoprenaline

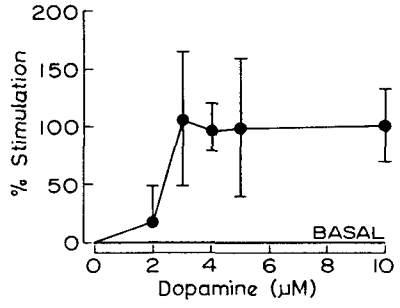

FIG. 1. Effect of dopamine on adenylate cyclase activity in homogenate of canine renal artery. Incubations were performed as described under Methods. Each point represents the mean ± s.e.m. of five experiments. The enzyme activity is expressed as percent stimulation over basal value. Stimulation by 4 μM dopamine was significantly different from the basal activity ($P < 0.05$). For other details refer to Table 1.

and dopamine effective in stimulating adenylate cyclase in the renal artery did not cause any stimulation of the enzyme activity in femoral artery. In fact, a five fold higher concentration of both drugs was needed to enhance the adenylate cyclase activity significantly in the femoral artery. The maximal stimulation produced by isoprenaline (0·2 μM) in the femoral artery was similar to that produced by the β-adrenoceptor agonist (0·04 μM) in the renal artery, whereas the stimulation elicited by dopamine was 3 times greater in the latter vessel.

Particulate fraction. The basal activity of adenylate cyclase in particulate preparations of renal arteries from untreated and phenoxybenzamine-treated dogs was twice that observed with homogenate preparation (See legends to Tables 1 and 2). Dopamine (4 μM) and (±)-isoprenaline (0·04 μM) stimulated the enzyme activity in renal arteries obtained from either group of animals (Table 2). A significant ($P < 0.05$) increase in the enzyme activity was produced by 4 μM dopamine (Fig. 2).

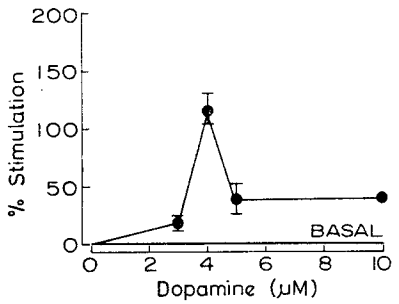

FIG. 2. Effect of dopamine on adenylate cyclase activity in particulate fraction derived from renal artery of phenoxybenzamine-pretreated dog. Each point represents the mean ± s.e.m. of five experiments and indicates the % stimulation over basal value. Stimulation by 4 μM dopamine was significantly different from the basal activity ($P < 0.05$). See Fig. 1 and Table 2 for other details.

The concentration of (±)-isoprenaline required for maximal stimulation was the same as in the case of homogenates, namely 0·04 μM (data not shown). Phenoxybenzamine pretreatment of dogs had no effect either on the basal enzyme activity or on the dopamine-stimulated enzyme activity of renal artery.

Effect of antagonists. Preliminary experiments were conducted to study the specificity of the dopamine-induced stimulation of enzyme activity in the renal

Table 2. *Adenylate cyclase activity in renal artery from untreated (control) and phenoxybenzamine-pretreated (treated) dogs.* Particulate fraction derived from renal artery served as the source of adenylate cyclase. Basal enzyme activity from the control and treated dogs were 7·40 ± 1·80 and 7·60 ± 1·20 units, respectively. Each experiment was performed in triplicate incubations.

Additions	Adenylate cylase activity (% basal) Control	Treated
None (basal)	100 ± 24 (8)*	100 ± 16 (7)
Dopamine, 4 μM	183 ± 18† (8)	230 ± 33† (7)
(±)-Isoprenaline, 0·04 μM	150 ± 34 (3)	155 ± 9† (6)

* Values in parentheses represent the number of experiments performed on different days.
† Significantly different from basal activity ($P < 0.05$).

Table 3. *Effect of agents on adenylate cyclase activities in the particulate fractions of renal and femoral arteries from phenoxybenzamine-treated dogs.* Basal enzyme activities of renal and femoral arteries were 10·60 ± 3·00 units and 9·95 ± 1·68 units respectively. Data presented are from a single experiment and represent the average of values obtained from triplicate incubations.

Additions	Adenylate cyclase activity (% basal) Renal	Femoral
None (basal)	100	100
Dopamine (4 μM)	175	—
Dopamine (20 μM)	—	179
(±)-Isoprenaline (0·04 μM)	164	—
(±)-Isoprenaline (0·20 μM)	—	160
Haloperidol (0·01 μM)	149	90
(±)-Propranolol (0·04 μM)	120	—
(±)-Propranolol (0·20 μM)	—	164
Dopamine (4 μM) + haloperidol (0·01 μM)	21	—
Dopamine (20 μM) + haloperidol (0·01 μM)	—	96
Isoprenaline (0·04 μM) + haloperidol (0·01 μM)	155	—
Isoprenaline (0·20 μM) + haloperidol (0·01 μM)	—	83
Dopamine (20 μM) + propranolol (0·04 μM)	181	—
Dopamine (20 μM) + propranolol (0·20 μM)	—	101
Isoprenaline (0·04 μM) + propranolol (0·04 μM)	28	—
Isoprenaline (0·20 μM) + propranolol (0·20 μM)	—	83

and femoral arteries of phenoxybenzamine-treated dogs. The effects of antagonists on the adenylate cyclase activity of both arteries are summarized in Table 3. In one experiment, where haloperidol was present together with dopamine, the enzyme activity in the renal artery was depressed below basal level. This effect appeared to be specific since haloperidol did not affect the isoprenaline-stimulated activity. Surprisingly the butyrophenone derivate alone stimulated the basal adenylate cyclase activity. Although propranolol also stimulated the basal enzyme activity, the β-adrenoceptor blocking agent antagonized the stimulation induced by isoprenaline without affecting the dopamine-induced stimulation. In contrast, both dopamine and isoprenaline-induced stimulation of enzyme activities in the femoral artery were abolished by haloperidol and propranolol.

DISCUSSION

Dopamine has the potential to act on several different receptors in the cardiovascular system. It has been shown (Goldberg, 1972; Toda & Goldberg, 1973) that dopamine-induced cardiac stimulation *in vivo* is antagonized by propranolol, suggesting a β-adrenoceptor mechanism, and that vasoconstriction caused by dopamine is blocked by large doses of phenoxybenzamine, suggesting also an α-adrenoceptor nature of dopamine action. In contrast, dopamine causes renal vasodilation by acting on an apparently different receptor (McNay & Goldberg, 1966). This effect is attenuated selectively by butyrophenones and phenothiazines, suggesting a similarity to putative dopamine receptors in the mamalian brain (Clement-Cormier, Kebabian & others, 1974).

Initial studies indicated that dopamine increased adenylate cyclase activities in homogenates of canine arteries. However wide variations in the concentration response curve suggested that the use of a more purified enzyme fraction would yield better reproducible results. Although isoprenaline was reported to increase adenylate cyclase activity in intact blood vessels, by earlier investigators, they were unable to demonstrate such a stimulation in homogenates of arterial tissues (Schonhoffer, Skidmore & others, 1971; Triner & others, 1972). In our particulate preparations, while the effect of each drug was dose dependent, the maximal stimulation occurred within a narrow range of concentrations. This may partially explain the earlier unsuccessful attempts to demonstrate isoprenaline stimulation of adenylate cyclase activity in homogenate preparations by other investigators.

Dopamine-induced stimulation of adenylate cyclase activity in the renal artery may not be related to an action on β-adrenoceptors, since concentrations of propranolol which inhibited the effect of isoprenaline did not affect dopamine-induced stimulation. The possibility that the action of dopamine is related to a stimulation of α-adrenoceptors can be ruled out, since the dogs were pretreated with phenoxybenzamine. However, the stimulation was selectively inhibited by haloperidol indicating that dopamine may exert a 'specific' stimulation of adenylate cyclase activity. In contrast, the dopamine-induced stimulation of the enzyme activity in femoral artery is inhibited by both propranolol and haloperidol at concentrations which blocked the isoprenaline-induced stimulation. These results suggest that the stimulatory effects of both dopamine and isoprenaline in the femoral artery are non-specific. Also, the results with femoral artery could not indicate whether the dopamine effect was due to the stimulation of specific receptors or not.

The limited studies reported here were aimed at demonstrating the *in vitro* stimulation of adenylate cyclase activity by dopamine using cell free preparations of arteries. It is possible that the dopamine-induced increase in adenylate cyclase activity in renal artery is related to a specific dopamine receptor. Additional studies are needed to confirm such a possibility and to explain satisfactorily the relationship between the putative dopamine receptor and the relaxation observed in renal artery.

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