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Comparison of the Effects of Dopamine and *Beta*-Adrenergic Agonists on Adenylate Cyclase of Renal Glomeruli and Striatum[1]

CONNIE KOTAKE,[2] PHILIP C. HOFFMANN, LEON I. GOLDBERG, AND JOSEPH G. CANNON

Departments of Pharmacological and Physiological Sciences and Medicine and the Committee on Clinical Pharmacology, The University of Chicago, Chicago, Illinois 60637, and College of Pharmacy, University of Iowa, Iowa City, Iowa 52242

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SUMMARY

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A dopamine-sensitive adenylate cyclase was identified in glomeruli prepared from rat kidney. The properties of central and peripheral cyclase-linked dopamine receptors were then compared by testing the ability of dopamine, isoproterenol, and the dopamine analogues, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (A-6,7-DTN), 2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene (A-5,6-DTN), and *N,N*-di-*n*-propyl dopamine (DPDA) to stimulate cyclic AMP production in homogenates and intact cell preparations of rat striatum and glomeruli. Cyclic AMP production in striatal homogenates was increased by dopamine, A-6,7-DTN, and DPDA, although DPDA was much less potent than the other two agonists. A-5,6-DTN (0–1000 μM) and isoproterenol (0–100 μM) were without effect in this preparation. In contrast, in a homogenate of kidney glomeruli as well as in striatal slices and in the intact glomeruli, all of these agonists increased cyclic AMP production. The pharmacological character of this response was further characterized by using the dopamine antagonist, fluphenazine, and the *beta*-adrenergic antagonist, propranolol. In glomerular homogenates, fluphenazine (10 μM) blocks the response to A-6,7-DTN (100 μM) and dopamine (100 μM) but does not affect the response to isoproterenol (1 μM) or A-5,6-DTN (100 μM). Propranolol (100 μM), on the other hand, blocked the response to isoproterenol, A-5,6-DTN, and partially blocked the dopamine response. A similar profile of activity was observed in intact glomeruli and in striatal slices. These findings suggest that both dopaminergic and *beta*-adrenergic receptors can mediate the production of cyclic AMP in the rat striatum and in the kidney vasculature. It is further suggested that dopamine can activate both types of receptors, depending on its concentration. The *beta*-rotameric conformer of dopamine (as exemplified by the semi-rigid analogue A-6,7-DTN) preferentially activates dopamine receptors, whereas the *alpha*-rotameric conformer (A-5,6-DTN) preferentially activates *beta*-adrenergic receptors. The potency series for activation of dopamine receptors is dopamine = A-6,7-DTN > DPDA in both tissues. The relative potency for activation of the *beta*-adrenergic response is also similar in both tissues. On the basis of the limited series of compounds studied, the cyclase-linked receptor in kidney vasculature and that in striatum appear to be quite similar.

INTRODUCTION

Dopamine-specific vascular receptors found in the renal, mesenteric, coronary, and cerebral vascular beds have been characterized by the vasodilation induced by a series of dopamine agonists. Additionally, it has been demonstrated that dopamine can interact with *alpha*- and *beta*-adrenergic vascular receptors (1). Study of semirigid tetrahydronaphthalene analogues of dopamine has allowed one to discriminate among the vascular responses mediated by dopamine-specific and *beta*-adrenergic receptors (2). In areas of the central nervous system innervated by dopaminergic neurons, dopamine receptors have been characterized by determination of a biochemical response: namely, dopamine stimulation of

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adenylate cyclase activity (3–5) as well as by radioactive ligand binding studies (6, 7).

We now report that the peripheral dopamine vascular receptor may also be characterized by means of a dopamine-sensitive adenylate cyclase localized in a rat glomerular preparation. This finding allowed us to compare more directly the properties of the central and peripheral dopamine receptors, since a common biochemical response to activation of the receptors can be measured.

MATERIALS AND METHODS

Tissue Preparation

Rat kidney glomeruli. Rat kidney glomeruli were prepared by a modification of the method of Fong and Drummond (8). The isolated kidney cortex was dissected and chopped twice with a Mickel chopper (Brinkman Instruments, Inc., Westbury, N. Y.) using a 250-μm excursion. The slices were then gently pressed through a Teflon mesh with 105-μm^2 openings. The resulting material was suspended in Krebs' buffer and washed by centrifugation at 67 × *g* in a Sorvall RC-2 centrifuge (SS-1 rotor) for 10 min. The resulting pellet was suspended in buffer and layered on a discontinuous sucrose gradient of 28, 40, 44, and 58 g/100 ml, and centrifuged at 100 × *g* for 20–30 min. The layers containing glomeruli were found between 40 and 58 g/100 ml. These were removed and washed two times in buffer by centrifugation. The purity of the preparation was monitored by light microscopy. It was found to be free of red blood cells and to consist of a homogeneous preparation of "tuft"-like structures of a size and shape consistent with those of rat glomeruli (Fig. 1).

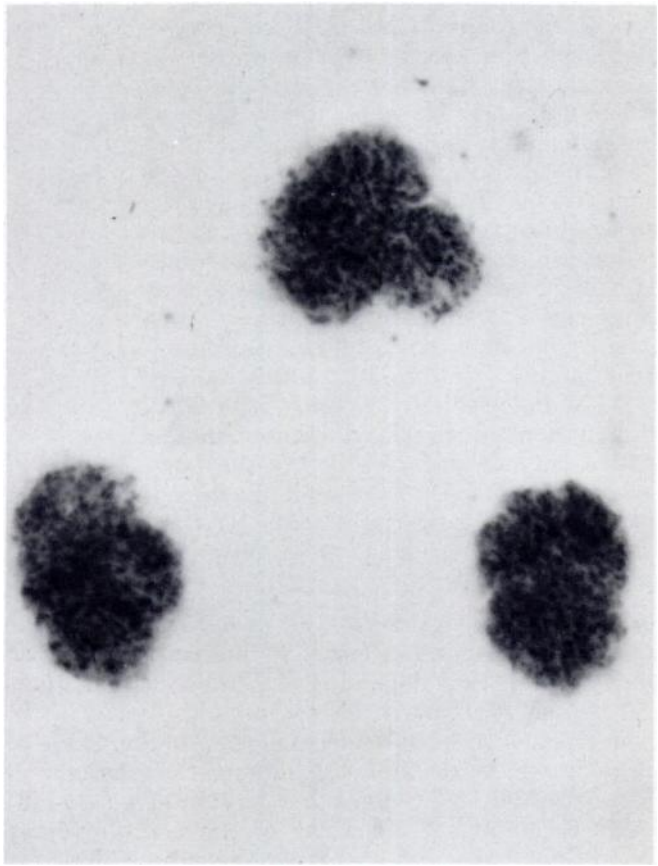

FIG. 1. *Photomicrograph of rat kidney glomeruli stained with Harns' hematoxylin and eosin(×260)*

Striatum. Male Sprague-Dawley rats were killed by decapitation and the striata were dissected. Striatal slices were prepared by chopping the striatum with a Mickel chopper (blade excursion 250 μm) and then further reducing the size by passing the slices through a mesh with 105-μm^2 openings to facilitate pipetting, as well as oxygenation and diffusion of drugs in the slices.

Both the adenylate cyclase activity of tissue homogenates and the accumulation of cyclic AMP in intact cells were determined. Adenylate cyclase activity was determined by the method of Kebabian *et al.* (3), except that 1.0 mM isobutyl-methyl xanthine was used and the incubation time was extended to 10 min for the glomerular homogenates.

Cyclic AMP accumulation from endogenous ATP in striatal slices or glomeruli was studied after resuspension in Krebs' buffer. Two hundred and fifty microliters of the suspension (containing 10–30 μg of protein) were incubated under 5% CO_2–95% O_2 for 10 min at 37°. The reaction was terminated by heating the tubes in a boiling water bath, particulate matter was removed, and protein was determined (9).

Cyclic AMP Determination

Antibody to 2′-*O*-succinyl cyclic AMP was prepared by the method of Steiner and co-workers (10). The antibody obtained did not cross-react with ATP in concentrations as high as 10^{-3} M. Significant cross-reactivity occurred only with cyclic GMP and 5′-AMP. The minimum detectable concentrations of these agents were 6×10^{-6} M and 3×10^{-2} M, respectively.

Aliquots of the supernatants obtained from the adenylate cyclase incubations were assayed for cyclic AMP content by a modification of the radioimmunoassay method of Steiner and co-workers (10). A similar procedure was followed for the determination of the cyclic AMP content in striatal slices or glomeruli following stimulation with various pharmacological agents, although the relatively lower levels of cyclic AMP produced under these conditions required radioimmunoassay of the acetylated cyclic AMP derivative (11).

The results were analyzed by Student's *t*-test comparison (two-tailed). The data shown represent three to five experiments; each experiment represents tissue from a single animal with duplicate cyclic AMP determinations.

Chemicals

Dopamine HCl and l-isoproterenol bitartrate (Sigma Chemical Company, St. Louis, Mo.); A-5,6-DTN[3] and *N,N*-di-*n*-propyl dopamine were prepared by J. G. Cannon; A-6,7-DTN was obtained from D. E. Nichols, Purdue University (Lafayette, Ind.); propranolol HCl (Ayerst Laboratories, New York, N. Y.); and fluphenazine HCl

[3] The abbreviations used are: A-5,6-DTN, 2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene; A-6,7-DTN, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

were obtained from Schering Corporation (Kenilworth, N. J.).

RESULTS

Rat glomeruli homogenates. Both dopamine and A-6,7-DTN elicited a dose-related stimulation of glomerular adenylate cyclase activity (Fig. 2). The EC_{50} for dopamine stimulation was about 50 μM. Maximal stimulation of about 75% was produced by 500 μM dopamine. The stimulation associated with the semirigid analogue A-6,7-DTN was about one-half that produced by dopamine throughout the concentration range. The basal adenylate cyclase activity had a value of 7.83 ± 0.76 pmoles/min/mg of protein.

The nature of the receptor(s) mediating this stimulation of adenylate cyclase activity was further investigated using the dopamine antagonist fluphenazine and the *beta*-adrenergic antagonist propranolol. Dopamine and A-6,7-DTN concentrations were set at 100 μM, which produced 75% of the maximal response for each agonist. The response to A-6,7-DTN was totally blocked by 10 μM fluphenazine (Fig. 3) but was unaffected by 10 μM propranolol. The response to dopamine was also virtually abolished by fluphenazine, but in contrast it was reduced by about 40% by propranolol (Fig. 3). Indeed, under conditions of propranolol blockade, the response to dopamine was essentially equal to that produced by an equimolar concentration of A-6,7-DTN.

The partial sensitivity of the dopamine response to propranolol suggested the possibility of a *beta*-adrenergic component in the response to this agonist. This possibility was further assessed with a *beta*-adrenergic agonist, isoproterenol, and the transoid, semirigid analogue of dopamine, A-5,6-DTN, at concentrations of 1 and 100 μM, respectively. These concentrations stimulated the enzyme activity by about 75%, essentially to the same extent as 100 μM dopamine. The response to both of these agonists was blocked by propranolol but unaffected by fluphenazine (Fig. 3). The stimulation of enzyme activity by the *N*,*N*-di-substituted dopamine analogue, dipropyl dopamine, was 33 ± 18% ($n = 3$) of that produced by an equimolar (100 μM) concentration of dopamine itself.

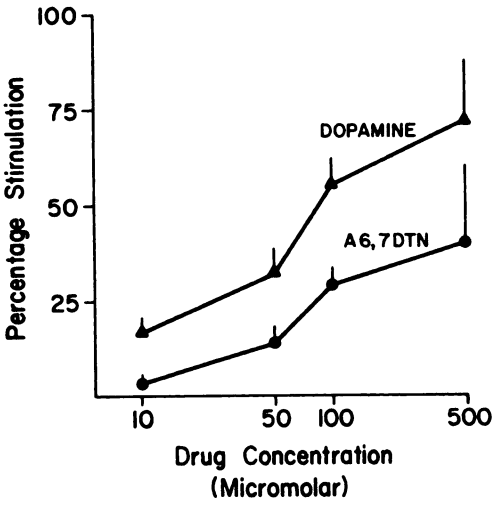

FIG. 2. *Effect of dopamine (▲) and A-6,7-DTN (●) on the adenylate cyclase activity of rat kidney glomerular homogenates*

Basal adenylate cyclase activity was 7.83 ± 0.76 pmoles/min/mg of protein ($n = 4$ preparations). Percentage stimulation represents the difference between the activity observed in the presence and absence of each agonist. The *error bars* represent the standard error of the mean.

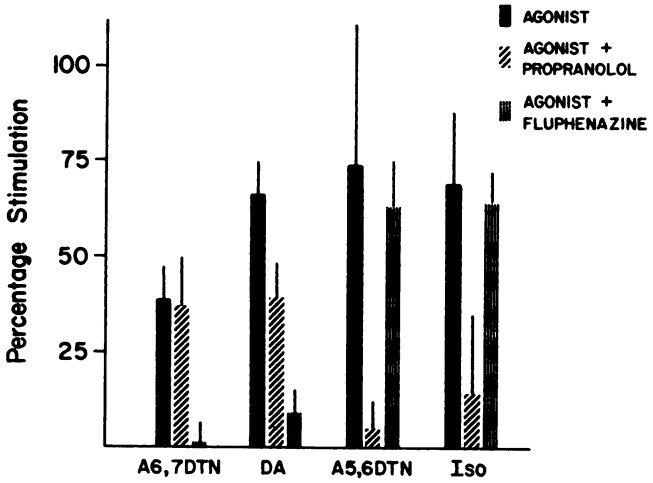

FIG. 3. *Effect of beta-adrenergic and dopaminergic agonists and antagonists on the adenylate cyclase activity of homogenates of rat kidney glomeruli*

Solid bars, no antagonist; *diagonally striped bars*, + 100 μM propranolol; *vertically striped bars*, + 10 μM fluphenazine. Agonist concentrations were as follows: A-6,7-DTN, 100 μM; dopamine, 100 μM; A-5,6-DTN, 100 μM; isoproterenol, 1 μM. Percentage stimulation represents the difference between the activity in the presence of agonist or of agonist plus antagonist and the activity in the absence of drugs. The *error bars* represent the standard error of the mean ($n = 4$ preparations).

Intact rat glomeruli. The effects of all of the agonists on the accumulation of cyclic AMP in a preparation of intact glomeruli were also studied. Basal cyclic AMP levels were 8 pmoles/mg of protein. The responses of the intact glomeruli to A-6,7-DTN and isoproterenol (Fig. 4) were very similar to the responses of the glomerular homogenates with respect to both agonist activity and antagonist effectiveness. However, propranolol antagonized the response to dopamine to a greater extent than was the case in the homogenates, and, conversely, fluphenazine was not as effective in blocking the dopamine-induced stimulation (Fig. 4).

Homogenates of rat striatum. The effects of dopamine, A-6,7-DTN, and A-5,6-DTN on adenylate cyclase activity were studied in striatal homogenates. The basal activity of the enzyme was 103 ± 5 pmoles/min/mg of protein. This basal activity was approximately 12 times that found in the glomerular homogenates. Equivalent stimulation occurred with dopamine and A-6,7-DTN in concentrations from 1 to 100 μM (Fig. 5). Compared with the glomerular preparations, considerable stimulation could be observed at lower agonist concentrations (1–10 μM). In contrast, A-5,6-DTN had no effect on striatal adenylate cyclase activity at concentrations as high as 1000 μM. The response of the enzyme to dipropyl dopamine was 56 ± 4% ($n = 4$) of the response to an equimolar (100 μM) concentration of dopamine itself.

Rat striatal slices. Because the striatal homogenates

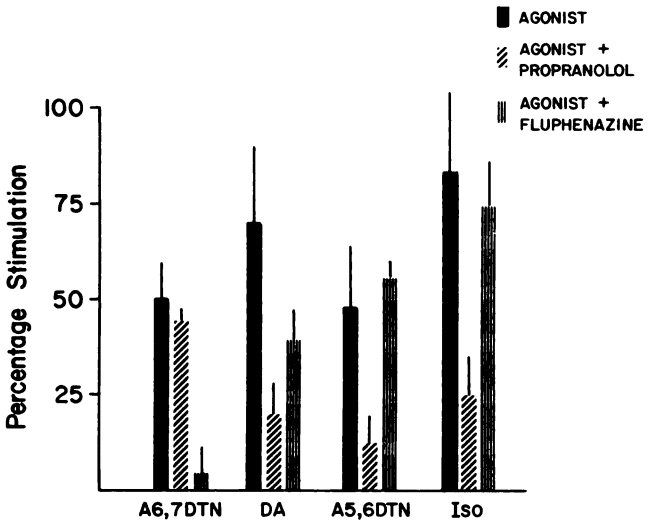

FIG. 4. *Effect of beta-adrenergic and dopaminergic agonists and antagonists on cyclic AMP accumulations in intact kidney glomeruli*

Basal cyclic AMP levels were 8.0 ± 0.1 pmoles/mg of protein (n = 4). *Solid bars*, no antagonist; *diagonally striped bars*, + 100 μM propranolol; *vertically striped bars*, + 10 μM fluphenazine. Agonist concentrations were as follows: A-6,7-DTN 100 μM; dopamine, 100 μM; A-5,6-DTN, 100 μM; isoproterenol. 1 μM. Percentage stimulation represents the difference between cyclic AMP levels observed in the presence of agonist or of agonist plus antagonist and the activity in the absence of drugs. The *error bars* represent the standard error of the mean (n = 4 preparations).

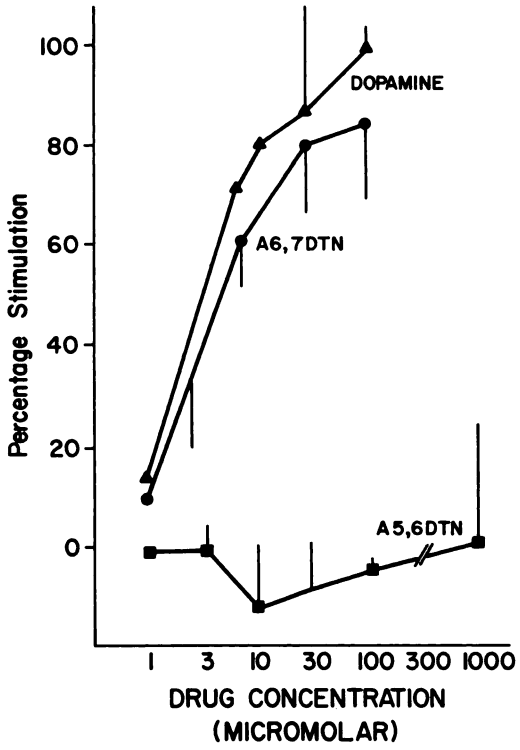

FIG. 5. *Stimulation of dopamine-sensitive adenylate cyclase in rat striatal homogenates by dopamine (▲), A-6,7-DTN (●), and A-5,6-DTN(■)*

Basal cyclase activity was 103 ± 5 pmoles/min/mg of protein (n = 5). Percentage stimulation represents the difference between the activity observed in the presence and absence of each agonist. The *error bars* represent the standard error of the mean.

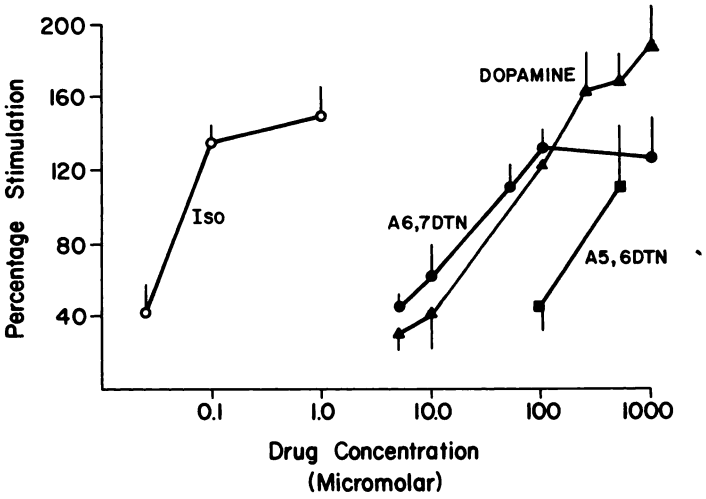

FIG. 6. *Effect of dopamine (▲), A-6,7-DTN (●), A-5,6-DTN (■), and isoproterenol (○) on cyclic AMP accumulation in rat striatal slices*

Basal levels were 3.0 ± 0.4 pmoles/mg protein (n = 8). Percentage stimulation represents the difference between cyclic AMP levels observed in the presence and absence of each agonist. The *error bars* represent the standard error of the mean.

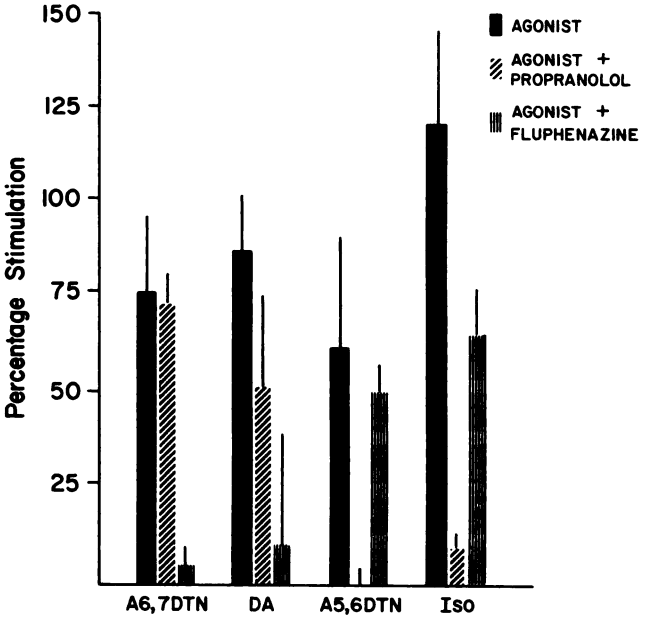

FIG. 7. *Effect of beta-adrenergic and dopaminergic agonists and antagonists on cyclic AMP accumulation in rat striatal slices*

Solid bars, no antagonist; *diagonally striped bars*, + 100 μM propranolol; *vertically striped bars*, + 10 μM fluphenazine. Agonist concentrations were as follows: A-6,7-DTN, 100 μM; dopamine, 100 μM; A-5,6-DTN, 100 μM; isoproterenol, 1 μM. Percentage stimulation represents the difference between cyclic AMP accumulation in the presence of agonist or of agonist and antagonist and cyclic AMP accumulation in the absence of drugs. The *error bars* represent the standard error of the mean.

differed from the glomerular homogenates in that they did not respond to the *beta*-adrenergic agonists, A-5,6-DTN or isoproterenol (12), all of the agonists were further compared for their ability to stimulate cyclic AMP accumulation in striatal slices containing intact cells. Basal levels of cyclic AMP were 3.0 ± 0.4 pmoles/mg of

protein. Isoproterenol was much more potent in stimulating cyclic AMP accumulation in the slices than were dopamine or the semirigid analogues of dopamine (Fig. 6). However, both dopamine and A-6,7-DTN were more potent than A-5,6-DTN. The effects of propranolol and fluphenazine on these responses are shown in Fig. 7. Propranolol reduced the mean response to dopamine by approximately 45%, but because of the considerable variability, this reduction was not statistically significant. The response to A-6,7-DTN was unaffected by propranolol. Fluphenazine abolished the effect of both dopamine and A-6,7-DTN. The response to *l*-isoproterenol was greater than that produced by 100 times as much A-5,6-DTN. Propranolol virtually eliminated the response to both A-5,6-DTN and isoproterenol, whereas fluphenazine (10 μM) did not significantly affect the responses to these *beta*-adrenergic agonists.

DISCUSSION

Previous studies of the dopamine vascular receptor have been largely confined to physiological measurements of changes in blood flow. A major goal of this investigation was to define a biochemical system responsive to dopamine in a peripheral vascular tissue. Such a system would facilitate comparison with dopamine receptors in the central nervous system. The present results suggest that dopamine-sensitive adenylate cyclase activity of isolated glomeruli from rat kidney may represent such a biochemical system. Previous investigators demonstrated the presence of a dopamine-sensitive cyclic AMP-generating system in kidney preparations (13, 14). However, both studies failed to show dose-dependent stimulation of dopamine-sensitive adenylate cyclase in homogenates, and the preparation used by Nakajima *et al.* (13) contained considerable amounts of nonvascular tissue.

The results suggest that glomeruli contain both dopaminergic and *beta*-adrenergic receptors which, when activated, stimulate cyclic AMP production. The dopaminergic receptor is revealed with A-6,7-DTN, the activity of which is unaffected by propranolol and, conversely, blocked by fluphenazine. The beta-adrenergic receptor is activated by isoproterenol and A-5,6-DTN, the semirigid analogue of dopamine, which is devoid of dopaminergic activity (2). The response to these agonists is blocked by propranolol but is unaffected by fluphenazine. Dopamine appears to behave as a mixed agonist with both a dopaminergic and a *beta*-adrenergic component, since the response to dopamine is consistently twice the response to an equimolar concentration of A-6,7-DTN (Fig. 2). Furthermore, the response to 100 μM dopamine is reduced by propranolol to the level of the response obtained with an equimolar concentration of A-6,7-DTN (Fig. 3). Correspondingly, in intact glomeruli, fluphenazine, the dopamine antagonist, blocks the response to dopamine by only 50%. Evidence contradicting the suggestion that dopamine behaves as a mixed agonist in this system is provided by the finding that fluphenazine in the cell-free preparation appears to block totally the response to dopamine. In order to resolve this question, complete dose-response data for dopamine in the presence of varying concentrations of the two antagonists are required.

In both striatal homogenates and slices, A-6,7-DTN, the *beta*-rotameric conformer of dopamine, stimulates cyclic AMP accumulation with the same potency as dopamine. This suggests that, as with the dopamine vascular receptor (2), the *beta*-rotamer is the preferred conformer of the dopamine molecule for activation of this striatal dopamine receptor. These results are in basic agreement with those of Miller *et al.* (4), Sheppard *et al.* (5), and Woodruff *et al.* (15).

While A-5,6-DTN, the *alpha*-rotameric conformer of dopamine, had no effect in the homogenate, it increased cyclic AMP accumulation in striatal slices, although its potency was about 10 times less than that of A-6,7-DTN. The receptors mediating the A-5,6-DTN response are probably *beta*-receptors, since this response was eliminated by propranolol but unaffected by fluphenazine. However, the potency of A-5,6-DTN in interacting with these receptors is about 3 orders of magnitude less than that of isoproterenol.

Given the fact that this semirigid conformer of dopamine can interact with *beta*-receptors at high concentrations, it is quite likely that the flexible dopamine molecule itself can interact with them. This suggestion supports the work of Minneman *et al.* (16), who showed that 300 μM dopamine enhances cyclic AMP accumulation in striatal slices, at least in part, through a *beta*-adrenergic mechanism. In our hands, propranolol reduced the mean response to 100 μM dopamine by about 40%, but, because of the large variability, this difference was statistically insignificant. We believe that the most reasonable interpretation of these data is that the affinity of dopamine for dopamine receptors is considerably greater than its affinity for the *beta*-adrenergic receptor. Thus, at the lower end of the dose-response curve (i.e., below 100 μM), cyclic AMP accumulation is stimulated largely by activation of dopamine receptors. However, at concentrations of 100 μM and greater, there is an increasing contribution to the cyclic AMP accumulation resulting from activation of *beta*-adrenergic receptors. One hundred micromolar appears to be the threshold concentration for activation of these *beta*-adrenergic receptors. This suggestion is supported by the fact that the semirigid analogue A-5,6-DTN first causes measurable stimulation of enzyme activity through a *beta*-adrenergic mechanism at a concentration of 100 μM. Such an interpretation would also help to explain the apparent biphasic nature of the dose-response curve to dopamine itself, in which concentrations of dopamine greater than 100 μM produce increases in cyclic AMP accumulation which are 40% greater than those produced by comparable concentrations of A-6,7-DTN.

A comparison of the properties of the dopamine receptors mediating the stimulation of adenylate cyclase in the isolated glomeruli and in the striatum show that they are quite similar. In both systems, the potency series is dopamine = A-6,7-DTN > dipropyl dopamine. A relatively minor difference in the potency concerns the relative activity of dopamine and A-6,7-DTN. These two agonists appear to be equipotent in striatal homogenates, whereas in the glomerular homogenates A-6,7-DTN

shows consistently one-half the activity of dopamine throughout the concentration range tested.

The rat glomerular preparation appears to be a reasonable model in which to study the structure-activity requirements of a peripheral dopamine receptor and, in addition, allows a direct comparison to be made with dopamine-mediated adenylate cyclase activation in the striatum of the same species, the rat. In this study, a small series of dopaminergic agonists was studied in both areas and similar activity profiles were observed in these tissues, suggesting that the structure-activity requirements of the dopamine receptor linked to adenylate cyclase in the peripheral vasculature are similar to those found in the striatum.

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Send reprint requests to: Dr. Philip C. Hoffmann, Department of Pharmacological and Physiological Sciences, University of Chicago, 947 East 58th Street, Chicago, Ill. 60637.