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Dopaminergic Inhibition of Vasopressin-Stimulated Water Flow in the Toad Bladder: Evidence for Local Formation of Dopamine

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Summary. Dopamine administration increases renal excretion of water and Na. It remains uncertain whether these effects of dopamine are the result of a hemodynamic effect or the consequence of a direct cellular action. We investigated the effect of dopamine on water transport by the isolated toad bladder *in vitro*. Dopamine failed to alter baseline water flow but caused a significant inhibition of arginine vasopressin (AVP) or cyclic adenosine monophosphate (AMP) stimulated water flow. The effect of dopamine on stimulated water flow was not due to activation of α adrenergic, β adrenergic, or cholinergic receptors. The selective antagonists of dopamine, metoclopramide and apomorphine, prevented the effect of dopamine on AVP-stimulated water flow. These observations suggest the existence of a dopaminergic receptor in the toad bladder. L-Dopa also inhibited AVP-stimulated water flow. The effect of L-Dopa could be prevented by metoclopramide, thus suggesting that L-Dopa is converted to dopamine by an aromatic amino acid decarboxylase present in the toad bladder. To investigate this possibility we measured the effect of the decarboxylase inhibitor, carbidopa, on the $^{14}CO_2$ production generated by decarboxylation of ^{14}C L-Dopa in isolated toad bladder epithelial cells. Isolated toad bladder epithelial cells generated significant amounts of $^{14}CO_2$ from ^{14}C L-Dopa. This effect could be blocked by carbidopa, thus suggesting the existence of an aromatic amino acid decarboxylase system in the toad bladder. Carbidopa also prevented the inhibitory effect of L-Dopa on AVP-stimulated water flow, suggesting that L-Dopa needs to be converted to dopamine to inhibit water flow. These data suggest the existence of a dopaminergic receptor in the toad bladder. These data also suggest that dopamine can be formed locally in the toad bladder and can thus serve as a local modulator of water transport.

Key words dopamine · water transport · toad bladder · vasopressin

Introduction

Cathecholamines have been shown to influence the transport of electrolytes and water *in vitro* and *in vivo* [28, 29]. Dopamine administration *in vivo* results in increased excretion of Na, H_2O, and other electrolytes [5, 7, 8, 11, 22, 24]. It remains uncertain whether these effects of dopamine *in vivo* result from a direct effect of the drug on transport processes or is the result of hemodynamic alterations caused by the neurotransmitter [14]. It has been demonstrated that dopamine may be formed locally in the kidney by an aromatic amino acid decarboxylase which is present in large amounts in the kidney [3, 9, 20, 25, 33]; dopamine could thus serve as a local modulator of certain transport processes.

The role of dopamine on water transport, however, remains to be completely defined. Previous studies in the toad bladder have suggested that dopamine is capable of inhibiting vasopressin-stimulated water flow [2, 30]. It is not known whether this effect of dopamine is related to activation of a specific dopaminergic receptor. Also unknown is whether the toad bladder is capable of forming dopamine from its percursor L-Dopa. In the present study we characterized the effect of dopamine on water transport by the toad bladder. Our results demonstrate that dopamine inhibits vasopressin-stimulated water flow probably by interacting with a dopaminergic receptor. These data also indicate that dopamine may be formed locally in the toad bladder and can thus serve as a modulator of transport.

Materials and Methods

Water Flow

Experiments were performed on bladders isolated from toads, *Bufo Marinus*, obtained from Rand McNally and said to originate from North America. The bladders were excised, divided into halves (one serving as control and the other as experimental) and mounted as sacs in glass tubing, as previously described [1]. The bladders were filled with 5 ml of diluted Ringer's solution (diluted 1:5) and suspended in a bath containing 100 ml of full-strength Ringer's containing the following composition (in mmol/liter): Na, 112; Cl, 115; K, 5.0; Ca, 1.0; HPO_4, 2.4; and H_2PO_4, 0.6; dextrose, 5 mM; pH 7.4, osmolality, 241 mOsm/kg H_2O. The bathing solu-

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tion in which the bladders were suspended was fully aerated with compressed air and vigorously stirred. Water flow was measured gravimetrically at 30-min intervals; the bladders were removed from the bathing solution, blotted gently, and weighed on a Mettler balance. After an equilibration period, two baseline weight measurements at 30-min intervals were obtained. After addition of one experimental agent to the serosal solution (or an equal amount of diluent to the control hemibladder), one or two additional weight measurements at 30-min intervals were obtained. All experiment agents were dissolved in the toad Ringer's and pH was adjusted to 7.4 before addition to the bathing solution of the bladder. The pH of the serosal solution was monitored continuously and was maintained at 7.4 throughout the experiments. Vasopressin (AVP, 20 mU/ml) or cyclic adenosine monophosphate (AMP, 10 mM) was added to the serosal solution of both control and experimental hemibladders; weight loss was measured 30 min after the addition of AVP or cyclic AMP.

A different protocol was utilized when examining the effect of dopamine on submaximal doses of AVP or cyclic AMP. In this protocol after baseline water flow measurements, both hemibladders were stimulated with AVP (1 mU/ml) or cyclic AMP (1 mM) or butyl-1-methylxanthine (MIX, 2 mM) (period I) for 30 min. The serosal solution was then washed twice, the bladders were refilled, and 90–120 minutes were allowed for the water flow to return to the original baseline values. Dopamine was then added to the serosal solution of one hemibladder, and the other hemibladder served as control. Thirty minutes after addition of dopamine both hemibladders were restimulated with same dose of AVP, cyclic AMP or MIX (period II). To be included in the analysis the response of two hemibladders in period I could not differ more than 15%. Weight changes were corrected for the surface area of hemibladders by assuming that each hemibladder represented a perfect and constant 5 ml sphere in all periods of the experiments; the results are expressed as the difference from the preceding value in $\mu l/cm^2/hr$ and are presented as mean ± SEM [1]. The data were analyzed by the paired "t" test.

L-*Dopa Decarboxylase in Toad Bladder Epithelial Cells*

A suspension of toad cells was prepared as previously described [1] and assayed for L-Dopa decarboxylase activity according to a modification of the method described by Lloyd and Hornykiewicz [19]. One half milliliter of cells (1.5–3.0 mg protein/ml) was placed in the outer well of an incubation flask containing toad Ringer's solution. The inner well contained 0.2 ml Hyamine. The reaction was started by the rapid injection of 2.5 mM L-Dopa containing ^{14}C-Dopa (sp act = 0.2 μCi/mM). The final volume was 2.0 ml, pH 7.4. The mixture was placed in a shaking water bath for 2 hr at 37 °C. One milliliter of 50% trichloroacetic acid was rapidly injected and allowed to incubate for an additional 45 min. At the end of the experiment the Hyamine was quantitatively transferred to a scintillation vial containing 10 ml Scintiverse® (Fischer Scientific) and counted. In experiments containing carbidopa (2.5 mM), the inhibitor was preincubated 15 min prior to the addition of ^{14}C-Dopa. The $^{14}CO_2$ liberated was calculated based on the original specific activity of ^{14}C-Dopa. Tissue blanks and reagent blanks were run with each incubation and appropriate corrections for quenching made. Protein determination was performed according to the method of Lowry et al. [21].

Dopamine, L-Dopa, atropine, apomorphine, D-I propanolol and cyclic AMP were obtained from Sigma Co., vasopressin from Parke Davis, phentolamine from Ciba. Carbidopa, I-(−)α-hydrazino-3, 4-dihydroxy-α-methylhydrocinnamic acid monohydrate was a gift from Merck, Sharp and Dohmne; metoclopramide was a gift from A.H. Robbins.

Table 1. Effect of dopamine on AVP or cyclic AMP-stimulated water flow

Drug	Dopamine concentration (M)	N	Stimulated water flow ($\mu l/cm^2/hr$)		
			Control	$P<$	Dopamine
AVP (20 mU/ml)	10^{-6}	13	116.7 ± 7.5	NS	106.0 ± 16.6
AVP (20 mU/ml)	5×10^{-6}	10	113.5 ± 6.9	0.005	87.1 ± 5.9
AVP (20 mU/ml)	10^{-5}	12	116.4 ± 6.8	0.005	96.8 ± 8.8
AVP (20 mU/ml)	10^{-4}	13	130.2 ± 9.6	0.02	104.9 ± 6.1
AVP (20 mU/ml)	10^{-3}	16	131.7 ± 9.9	0.001	74.4 ± 11.8
Cyclic AMP (10 mM)	10^{-4}	6	107.2 ± 8.2	0.02	73.9 ± 6.6

Dopamine was added to the serosal solution 30 min before the addition of AVP (20 mU/ml) or cyclic AMP (10 mM) to the serosal solution. Stimulated water flow refers to values obtained 30 min after addition of AVP or cyclic AMP.

Results

Effect of Dopamine on AVP-Stimulated Water Flow

Table 1 shows the effect of various concentrations of dopamine, on AVP (20 mU/ml) or cyclic AMP (10 mM) stimulated water flow. Dopamine was added to serosal solution 30 min before the addition of AVP or cyclic AMP. Baseline water flow was not altered by dopamine (data not shown). At 10^{-6}M dopamine failed to alter AVP-stimulated water flow. At greater concentrations dopamine caused a significant inhibition of AVP or cyclic AMP-stimulated water flow. At comparable concentrations the magnitude of the inhibitory effect of AVP or cyclic AMP-stimulated water flow was very similar (Table 1).

Table 2 shows the effect of dopamine on submaximal dose of AVP (1 mU/ml), cyclic AMP (1 mM) or MIX (2 mM) to stimulate water flow in the toad bladder. In period I, both hemibladders served as controls and in period II, one hemibladder served as control and the other was treated with dopamine. Observe the close response of both hemibladders to AVP during period I. Notice also that the second stimulation with AVP in the control hemibladder yields a response almost identical to the first one. A 10^{-7}M dopamine caused a small, but insignificant, decrease in AVP-stimulated water flow. AVP-stimulated water flow was inhibited 18.5% at 10^{-6}M, 37% at 10^{-5}M, 45% at 10^{-4}M and 53% at 10^{-3}M dopamine. The effect of 1 mM cyclic AMP or 2 mM MIX to stimulate water flow was also inhibited by dopamine 10^{-4}M.

Table 2. Effect of dopamine on water flow stimulated by AVP (1 mU/ml), cyclic AMP (1 mM) or butyl-1-methylxanthine (2 mM)

Drug	Dopamine concentration (M)	N		Stimulated water flow (μl/cm²/hr)		Ratio of water flow II/I (%)	Inhibition[a] (%)
				Period I (control)	Period II (dopamine present in one bladder)		
AVP	Control	9		62.1±11.5	58.5±10.8	94.0±5.4	
			$P<$	NS	NS	NS	14
(1 mU/ml)	D-10^{-7}			58.1±10.0	50.4±9.6	87.0±10.4	
AVP	Control	8		35.3±5.4	34.2±4.1	97.0±5.1	
			$P<$	NS	0.001	0.001	18.5
(1 mU/ml)	D-10^{-6}			35.7±6.9	27.9±4.4	78.0±8.0	
AVP	Control	12		53.4±8.3	48.5±6.6	91.0±5.4	
			$P<$	NS	0.001	0.001	37
(1 mU/ml)	D-10^{-5}			50.9±7.5	30.7±4.2	60.0±5.0	
AVP	Control	10		79.3±11.9	75.3±10.2	95.5±5.8	
			$P<$	NS	0.001	0.001	45
(1 mU/ml)	D-10^{-4}			74.2±10.6	41.4±6.5	56.0±7.7	
AVP	Control	7		62.1±14.3	53.5±15.0	85.4±6.7	
			$P<$	NS	0.025	0.001	53
(1 mU/ml)	D-10^{-3}			68.9±15.8	25.5±9.6	37.0±5.8	
Cyclic AMP	Control	6		45.1±6.7	34.4±5.2	76.6±3.5	
			$P<$	NS	0.02	0.02	22
(2 mM)	D-10^{-4}			46.8±6.4	26.9±5.8	59.9±4.5	
MIX	Control	7		9.7±0.95	10.5±1.1	108.3±5.4	
			$P<$	NS	0.05	0.05	34
(2 mM)	D-10^{-5}			10.3±1.6	6.9±0.52	67.3±7.9	

In period I both bladders were stimulated either with AVP, cAMP or MIX. The drugs were then washed, and water flow was allowed to return to baseline state. 90 to 120 minutes later dopamine was added to serosal solution of one hemibladder and 30 min later AVP or cyclic AMP or MIX was added to both hemibladders.

[a] Inhibition refers to % ↓ AVP-stimulated water flow before and after addition of dopamine.

Effect of Dopamine on AVP-Stimulated Water Flow in Presence of Blockade of α-Adrenergic, β-Adrenergic, or Cholinergic Receptors

Dopamine is capable of stimulating α-adrenergic, β-adrenergic, and cholinergic receptors in addition to the dopaminergic receptors [14, 16]. To examine whether the effect of dopamine on AVP-stimulated water flow was mediated through α-adrenergic, β-adrenergic, or cholinergic receptor interaction, we pretreated one set of hemibladders with the appropriate antagonist and 30 min later added dopamine to both sets of hemibladders. Table 3 shows that α-adrenergic inhibition by phentolamine, β-adrenergic inhibition by propanolol, or cholinergic inhibition by atropine did not alter the effect of dopamine on AVP-stimulated water flow. The inhibitory effect of 10^{-4}M dopamine on Table 3 was of the same magnitude as that shown in Table 1. Phentolamine, atropine, and propranolol by themselves did not alter AVP-stimulated water flow.

Effect of Dopamine on AVP-Stimulated Water Flow in Presence of Dopaminergic Antagonists

Phenothiazines and haloperidol have been shown to antagonize the effect of dopamine in several tissues [4, 15, 16, 34]. At concentrations capable of antagonizing the effect of dopamine in other tissues, fluphenazine and haloperidol caused a profound inhibition of AVP-stimulated water flow in control hemibladders. It was thus impossible to use these compounds as antagonists of dopamine in the toad bladder.

Metoclopramide is a selective antagonist of dopamine in many tissues [10, 12, 15, 16, 23, 26, 31]. At 10^{-4}M metoclopramide caused a small inhibitory effect of AVP-stimulated water flow in control hemibladders (Table 4). The ability of metoclopramide to antagonize the effect of dopamine was examined by pretreating one set of hemibladders with metoclopramide 30 min before the addition of dopamine to both sets of hemibladders. Metoclopramide significantly inhibited the effect of dopamine on AVP stimulated

Table 3. Effect of phentolamine, atropine and propanolol on the inhibitory effect of dopamine on AVP-stimulated water flow

Drug	Concentration (M)	N	AVP-stimulated water flow (μl/cm²/hr) P<		
			Dopamine		Phentholamine + Dopamine
Dopamine + Phentolamine	10^{-4} 10^{-4}	10	92.8 ± 6.9	NS	87.8 ± 9.3
			Dopamine		Atropine + Dopamine
Atropine + Dopamine	10^{-4} 10^{-4}	18	85.0 ± 11.3	NS	100.6 ± 23.7
			Dopamine		Propanolol + Dopamine
Propanolol + Dopamine	10^{-4} 10^{-4}	11	83.5 ± 10.9	NS	101.5 ± 11.5
			Control		Phentolamine
Phentolamine	10^{-4}	9	107.4 ± 12.4	NS	129.6 ± 16.5
			Control		Atropine
Atropine	10^{-4}	6	117.0 ± 8.8	NS	120.4 ± 21.0
			Control		Propanolol
Propanolol	10^{-4}	11	113.9 ± 6.7	NS	97.3 ± 6.6

Phentolamine, propanolol or atropine were added to the serosal solution 30 min before the addition of dopamine. AVP (20 mU/ml) was added 30 min after the addition of dopamine.

Table 4. Effect of dopamine on AVP-stimulated water flow in presence of metoclopramide or apomorphine

Drug	Concentration (M)	N	AVP-stimulated water flow (μl/cm²/hr) P<		
			Dopamine		Metoclopramide + Dopamine
Dopamine + Metoclopramide	10^{-4} 10^{-4}	10	80.1 ± 7.96	0.025	122.6 ± 14.8
			Dopamine		Metoclopramide + Dopamine
Dopamine + Metoclopramide	10^{-5} 10^{-4}	5	80.6 ± 14.6	0.025	112.9 ± 18.0
			Control		Metoclopramide
Metoclopramide	10^{-4}	18	119.2 ± 7.1	0.05	103.1 ± 5.1
			Control		Apomorphine
Apomorphine	10^{-4}	11	104.9 ± 5.3	NS	108.8 ± 8.6
			Dopamine		Apomorphine + Dopamine
Apomorphine + Dopamine	10^{-4} 10^{-4}	16	78.9 ± 4.9	0.005	107.7 ± 7.9

Metoclopramide or apomorphine was added to the serosal solution 30 min before the addition of dopamine. AVP (20 mU/ml) was added 30 min after the addition of dopamine.

water flow. The values of AVP stimulated water flow in bladders treated with metoclopramide and dopamine were not significantly different from that of control hemibladders. Metoclopramide (10^{-4}M) also inhibited the effect of dopamine on water flow stimulated by 1 mU/ml of AVP (dopamine 10^{-5}M 34.7 ± 5.8, metoclopamide 10^{-4}M 56.7 ± 7.5 μl/cm²/hr, $P < 0.001$, $n = 9$).

Apomorphine has been shown to function either as dopamine antagonist or as dopamine agonist in different tissues [16]. Apomorphine failed to alter AVP stimulated water in control hemibladders but prevented the inhibitory effect of dopamine on AVP stimulated water flow (Table 4). Thus, in the toad bladder apomorphine acts as a dopamine antagonist. AVP stimulated water flow in bladders treated with dopamine and apomorphine was not different from that of control bladders.

Table 5. Effect of L-Dopa on vasopressin-stimulated water flow

Drug	Concentration (M)	N	AVP-stimulated water flow (μl/cm²/hr) P<		
			Control		L-Dopa
L-Dopa	10^{-6}	8	110.6±6.6	NS	101.2±9.6
			Control		L-Dopa
L-Dopa	10^{-5}	13	131.3±11.3	0.025	115.0±12.1
			Control		L-Dopa
L-Dopa	10^{-4}	14	122.3±8.8	0.01	97.8±10.5
			Control		L-Dopa
L-Dopa	10^{-3}	12	106.8±6.8	0.005	90.8±4.2
			L-Dopa		Metoclopramide+L-Dopa
L-Dopa+ Metoclopramide	10^{-4} 10^{-4}	11	52.1±8.0	0.001	97.0±11.3

L-Dopa was added to the serosal solution 30 min before the addition of AVP (20 mU/ml). Metoclopramide (serosal solution) was added 30 min before the addition of L-Dopa.

Table 6. $^{14}CO_2$ generation by isolated toad bladder epithelial cells incubated with ^{14}C L-Dopa

$^{14}CO_2$ (nmol/mg protein/2 hr)		
^{14}C L-Dopa	P<	^{14}C L-Dopa+Carbidopa
6.49±0.423 (n=12)	<0.01	1.73±0.547 (n=6)

Toad bladder epithelial cells incubated with 2.5×10^{-3} L-Dopa or with 2.5×10^{-3}M carbidopa and 2.5×10^{-3}M L-Dopa. Carbidopa was added to the suspension of epithelial cells 1 hr before the addition of L-Dopa.

Effect of L-Dopa on AVP-Stimulated Water Flow

L-Dopa failed to alter baseline water flow but caused a significant inhibition of AVP-stimulated water flow when added to the serosal solution in concentrations greater than 10^{-6}M (Table 5). The effect of L-Dopa on AVP-stimulated water flow could be antagonized by metoclopramide, suggesting that the effect of L-Dopa is mediated by its conversion to dopamine by L-Dopa decarboxylase system present in the toad bladder.

Effect of L-Dopa Decarboxylase Inhibitor, Carbidopa, on L-Dopa Decarboxylation and L-Dopa Inhibition of Water Flow

^{14}C L-Dopa incubated with isolated epithelial toad bladder cells was decarboxylated, as demonstrated by the generation of $^{14}CO_2$ [19]. Carbidopa was able to inhibit significantly the decarboxylation of L-Dopa [32]. The yield of $^{14}CO_2$ in the presence of carbidopa was not significantly different from that observed in a blank incubation without epithelial cells (Table 6).

Table 7. Effect of aromatic amino acid decarboxylase inhibitor, carbidopa, on L-Dopa inhibition of AVP-stimulated water flow

Drug	Concentration (M)	N	AVP-stimulated water flow (μl/cm²/hr) P<		
			Control		Carbidopa
Carbidopa	10^{-5}	8	114.0±9.6	NS	90.0±9.6
			Carbidopa+ L-Dopa		L-Dopa
Carbidopa+ L-Dopa	10^{-5} 10^{-5}	14	103.1±10.5	0.025	77.2±8.2
			Carbidopa+ dopamine		Dopamine
Carbidopa+ Dopamine	10^{-5} 10^{-5}	21	79.7±6.1	NS	75.4±46

Carbidopa was added to the serosal solution 1 hr before the addition of L-Dopa. AVP (20 mU/ml) was added 30 min after addition of L-Dopa.

Table 7 shows the effect of carbidopa on the inhibitory effect of L-Dopa on AVP-stimulated water flow. Carbidopa caused a small, though not significant, inhibitory effect of AVP-stimulated water flow in control hemibladders. To test whether carbidopa could prevent the effect of L-Dopa the decarboxylase inhibitor was added to one set of hemibladders 60 min before the addition of L-Dopa which was added to both sets of hemibladders. Carbidopa significantly decreased the inhibitory effect of L-Dopa on AVP-stimulated water flow. To demonstrate whether the effect of carbidopa was specific for L-Dopa we exam-

ined whether carbidopa could alter the inhibitory effect of dopamine on AVP-stimulated water flow (Table 7). Carbidopa failed to prevent the inhibitory effect of dopamine on AVP-stimulated water flow.

Discussion

Dopamine has been shown to increase renal excretion of sodium and water [8, 11, 22, 24]. It is unclear, however, whether these effects of dopamine are the result of hemodynamic changes or the consequence of a direct action of the drug. For this reason we examined the effect of dopamine on water transport by the isolated toad bladder *in vitro*. Our data demonstrate that dopamine is capable of inhibiting water flow stimulated by maximal and submaximal doses of vasopressin. The ability of dopamine to inhibit AVP-stimulated water flow was more striking when 1 mU/ml of AVP was used as compared to 20 mU/ml. When 1 mU/ml of AVP was utilized dopamine was able to inhibit AVP-stimulated water flow in a concentration as low as 10^{-6}M and exerted an increasingly inhibitory effect when higher concentrations were utilized. At higher concentrations of AVP, an inhibitory effect of dopamine was disclosed only at concentrations of 10^{-5}M or higher. The effect of dopamine on submaximal doses of AVP (Table 2) of the present study is very similar to those reported by Bentley et al. [2]. Our observations differ from those of Bentley et al. [2], in that dopamine inhibited both AVP and cyclic AMP-stimulated water flow whereas Bentley found only inhibition of AVP-stimulated water flow. The reason for this difference is unclear, but it may be due to a different experimental design. In the present study, the experiments utilizing cyclic AMP were paired before and after addition of dopamine (*see* Table 2), and this may have allowed detection of the small inhibitory effect of dopamine on cyclic AMP-stimulated water flow.

Because dopamine could inhibit AVP and cyclic AMP-stimulated water flow by activating phosphodiesterase and thus lead to break down of cyclic AMP, we measured whether dopamine would inhibit the effect of phosphosdieterase inhibitor, butyl-1-methylxanthine (MIX), on water flow. Dopamine significantly inhibited the effect of MIX to stimulate water flow, suggesting that phosphodiesterase activation is not the mechanism for the inhibitory effect of dopamine. These studies add further support to our finding that the inhibitory effect of dopamine occurs at a step beyond the generation of cyclic AMP.

Since dopamine is capable of interacting with various receptors, including the α-adrenergic, β-adrenergic, and cholinergic receptor of various organs, it was necessary to exclude the possibility that the effect of this drug on vasopressin-stimulated water flow was not the result of interaction of dopamine with those receptors [14]. By the use of selective antagonists of α-adrenergic, β-adrenergic, and the cholinergic receptors it became clear that the effect of dopamine could not be accounted for by interaction with these receptors. In this regard, our observations differ from previous studies [2], which have suggested that the effect of dopamine is mediated by activation of the α-adrenergic receptor. The reason for this difference is unclear.

These observations suggested that the effect of dopamine to inhibit vasopressin-stimulated water flow was the result of interaction of dopamine with a "specific" dopaminergic receptor in the toad bladder. Various antagonists of dopamine have been utilized to determine whether the effect of dopamine on various organs results from a specific interaction with a dopaminergic receptor [4, 6, 10, 12, 15, 16, 23, 26, 31, 34]. In the toad bladder, fluphenazine and haloperidol [15, 16, 34], two commonly used antagonists of dopamine, caused a profound inhibition of water transport. These compounds, therefore, could not be used as antagonists of the inhibitory effect of dopamine on water transport.

Metoclopramide, a drug thought to be a selective antagonist of dopamine in several systems, was then investigated [4, 10, 12, 15, 16, 23, 26, 31, 34]. Metoclopramide *per se* caused a small inhibitory effect of vasopressin-stimulated water flow. Despite this small inhibitory effect, metoclopramide was capable of preventing the inhibitory effect of dopamine on water transport. These observations suggest that the effect of dopamine is the result of a specific interaction with a dopaminergic receptor. To further characterize the effect of dopamine on water transport we utilized apomorphine, a drug which has been well characterized to function either as agonist or as an antagonist of dopamine receptors, depending on the system utilized. In the toad bladder apomorphine alone had no inhibitory effect on vasopressin-stimulated water flow but was capable of preventing the inhibitory effect of dopamine on vasopressin-stimulated water flow. These data provide strong support for the contention that the inhibitory effect of dopamine is mediated through a specific dopaminergic receptor.

Studies by Landuron et al. [17] and others [13, 18] in neural tissue have shown that spiroperone is an ideal ligand for studying dopamine receptors because of its high specific activity, high affinity ($< 10^{-9}$M) for specific binding sites and slow dissociation from receptors. Attempts to identify a dopaminergic receptor in the toad bladder were unsuccessful due to the high amount of nonspecific irreversible binding.

To examine whether dopamine can be formed locally in the toad bladder, we examined the effect of L-Dopa on water transport and measured the decarboxylation of L-Dopa to dopamine as assessed by generation $^{14}CO_2$ from ^{14}C L-Dopa [19]. These studies demonstrate that L-Dopa is capable of inhibiting water transport in the toad bladder. The effect of L-Dopa on water transport could be blocked by metoclopramide, an antagonist of dopamine, thus suggesting that the effect of L-Dopa is mediated by its conversion to dopamine. This contention is further supported by the studies showing that isolated toad bladder epithelial cells are capable of decarboxylating ^{14}C L-Dopa as assessed by the generation of $^{14}CO_2$ [19]. Carbidopa is an inhibitor of aromatic amino acid decarboxylase and has been shown to antagonize the effect of L-Dopa on various tissues [32]. Our studies demonstrate that carbidopa was capable of inhibiting decarboxylation of ^{14}C L-Dopa. Carbidopa alone had a small inhibitory effect on vasopressin-stimulated water flow. Despite this inhibitory effect carbidopa significantly decreased the inhibitory effect of L-Dopa on water flow. These data strongly suggest that effect of L-Dopa on water transport is mediated by its conversion to dopamine by a decarboxylase system present in the toad bladder.

The concentrations of dopamine required to elicit an effect are higher in the toad bladder than in the brain. The fact, however, that dopamine may be formed locally in the toad bladders suggests that critical concentration of the neurotransmitter may be achieved *in situ* to elicit a physiologic response.

The kidney has been shown to possess an aromatic amino acid decarboxylase capable of forming dopamine [3, 9, 20, 25, 27, 33]. If the observation of the present study can be extrapolated, they would suggest that locally formed dopamine in the kidney may be capable of inhibiting water transport.

In conclusion, the present study demonstrates that dopamine is capable of inhibiting water transport by the toad bladder. The data strongly suggest that dopamine can be formed locally in the toad bladder.

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