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Inhibition of the Permeability Response to Vasopressin and Oxytocin in the Toad Bladder:

Effects of Bradykinin, Kallidin, Eledoisin, and Physalaemin

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Summary. It has been shown by means of Bentley's *in vitro* preparation of the isolated urinary bladder of the toad, *Bufo marinus paracnemis* Lutz, that bradykinin reversibly inhibited the increase brought about by vasopressin on the permeability to water of the toad bladder. The increased hydro-osmotic response of the bladder to oxytocin was also inhibited by the kinin. The effect on water permeability was observed when bradykinin was added either to the serosal Ringer's solution or to the mucosal solution. The addition of bradykinin alone did not alter the basal osmotic water transfer across the bladder. In this context, bradykinin acted as a competitive antagonist of vasopressin (and oxytocin). Although lacking intrinsic activity, bradykinin exhibited affinity for receptor sites that are also common to the neurohypophysial hormones, causing a parallel shift of the log-dose/response curve for vasopressin without changing the maximal responses. The effects of other kinins (namely kallidin, eledoisin and physalaemin) on the toad bladder were also tested. Each of these drugs alone did not change the basal water flux across the bladder wall. Like bradykinin, these peptides inhibited the increase in water permeability evoked by vasopressin and oxytocin in the bladder. In view of the importance of neurohypophysial hormones and their target tissues to the osmotic homeostasis of amphibians, and the observation of antagonism between the kinins and the pituitary hormones coupled to the abundance of kinins in the amphibian organism, particularly in the skin and urinary bladder, teleological reasoning predicts a physiological role for the kinins, possibly functioning to dampen excesses and oscillations in membrane permeability that could occur in face of a constant and variable secretion of neurohypophysial hormone, thus adding to the homeostatic response of the amphibian organism.

Kinins are naturally occurring hormones widespread in the animal kingdom. They have been reported occurring in blood plasma of various species, including man (Diniz & Carvalho, 1963; Erdös, Miwa & Graham, 1967; Ankier & Starr, 1967; Zacest & Mashford, 1967), and have also been extracted from glandular tissues (Erspamer, 1949; Anastasi & Erspamer, 1963; Chiang, Erdös, Miwa, Tague & Coalson, 1968; Pisano, 1968), as well

as from the skin of anuran amphibians (Erspamer, Bertaccini & Cei, 196 1964, 1966; Anastasi, Erspamer & Bertaccini, 1965; Anastasi, Erspame Bertaccini & Cei, 1966) and from the urinary bladder of the toad (Furtad *in press*). In previous studies it has been established that bradykinin inhibi the increase produced by vasopressin and oxytocin in the permeability t water of isolated toad bladders (Furtado & Machado, 1966; Furtado, 196 as well as the increased sodium transport across isolated toad skins cause by vasopressin (Furtado & Machado, 1966).

The purpose of the present study was to examine other kinin-hormone namely kallidin, eledoisin and physalaemin, for their possible inhibitor action upon the permeability response of the toad bladder to neurohyp physial hormones. It will be shown that all these kinins inhibited th hormones in a manner much like that of bradykinin, i.e., presumably actin as competitive antagonists to vasopressin.

Materials and Methods

Animals

Toads (*Bufo marinus paracnemis* Lutz) were captured in the vicinity of Ribeirão Prêt São Paulo, Brazil, and kept at ambient temperature in a natural aquatic environmen The toads weighed from 400 to 750 g and were used within one week of arrival.

Urinary Bladder Preparation

A detailed description appeared previously (Furtado, 1967), similar to that describe by Bentley (1958). After the toad was pithed, its bilobed bladder was divided into tw separate sacs. Each lobe of the bladder was tied to a piece of plastic tubing, the seros side facing outwards. Each of the sacs was filled with 2.5 ml of diluted Ringer's solutio (about 50 mosm) and suspended in a chamber containing 10 ml of aerated norm Ringer's solution (about 200 mosm) at 25 ± 1 °C. Composition of the Ringer's solutio (mM) was: NaCl 111; KCl 4; $CaCl_2$ 2; $NaHCO_3$ 2.5; glucose 5.5; the pH of the solutio was 7.4 to 7.6. All glass material utilized in these experiments was siliconized glass.

Experimental Procedure

Net movement of water along the osmotic gradient was estimated gravimetrically a 20-min intervals by recording the weight loss during this period on a highly dampe balance. Both the mucosal and serosal solutions were then removed and replaced wit fresh Ringer's solutions. The test drugs were then added to the serosal bath for a 20-mi experimental period. Then, fresh solutions were reintroduced into both chamber an bladder for another 20 min, and this procedure was repeated in order to wash out drug and to allow time for recovery from experimental manipulations. Another experimenta period was then run with fresh solutions and the test substances. The procedure wa repeated after the washing periods. Up to three experimental periods were carried ou thus far. In each of these periods, a bladder sac was treated with the neurohypophysia

hormone and the other hemibladder was treated with the hormone and the antagonist. These experimental periods were arranged so as to exhibit an increasing contraction of hormone from one to another period and to show an alternating treatment with hormone plus antagonist for every hemibladder.

Calculations

The relative inhibitory potency of the kinins was calculated using those segments of the log-dose/response curves which were apparently linear and without appreciable deviation from parallelism. In other words, the linear phase of hormone-receptor interaction was utilized where the biological activity is directly proportional to the log concentration of the hormone.

Drugs

The drugs used were bradykinin (BRS-640, Sandoz), kallidin (KL-689, Sandoz), eledoisin (ELD-950, Sandoz), physalaemin (FI-6422, Farmitalia), oxytocin (Syntocinon, Sandoz), vasopressin (Pitressin, Parke-Davis), and angiotensin (Hypertensin, Ciba). The same bath of each peptide was used throughout the work.

Results

The basal water loss during control (hormone-free) periods in 100 different hemibladders varied from 0.5 to 2.5 mg/min, with a mean value ($\pm$s.e.) of 1.65 ± 0.4 mg/min.

The estimations of water loss by the bladder in response to neurohypophysial peptides were made using 20-min intervals, because recent work (Furtado, 1968) has shown that within this period, under the present experimental conditions, peak flow of water is reached (Fig. 1). Fig. 1 also shows that there was a correlation in the rate of fading of the response to the various concentrations of hormone: the higher the dose, the faster the decline in the response. These findings are essentially in concert with the results of Eggena, Schwartz and Walter (1968), despite some differences in the experimental preparation.

Previous studies (Furtado, 1967, 1968) have shown that angiotensin-II (9.5×10^{-7} M), bradykinin (9.5×10^{-7} M), kallidin (8.5×10^{-7} M), eledoisin (8.5×10^{-7} M), and physalaemin (8.0×10^{-7} M), when applied to the serosal side of the bladder, did not significantly alter the normal rate of osmotic water transfer across the organ.

Effects of Kinins in the Presence of Neurohypophysial Hormones

Angiotensin. Water loss in response to 2.6×10^{-9} M vasopressin[1] in five single pairs of bladders had an average value ($\pm$s.e.) of 11.5 ± 2.23 mg/min.

1 The molar concentration of vasopressin is only an approximate value since the hormone utilized in this work was a commercial preparation containing arginine-vasopressin as well as lysine-vasopressin.

This response was essentially unaffected by angiotensin-II (9.5×10^{-7} M) when both drugs were applied to the serosal side of the bladder, averaging 11.2 ± 2.14 mg/min.

Bradykinin. The increase in water transfer across the bladder in the presence of vasopressin was reduced when bradykinin (9.5×10^{-7} M) was added also (Table 1). The same inhibition was observed when the kinin was in contact either with the serosal or the mucosal surface of the bladder (Table 2). These effects were obtained without preincubation of the bladder with bradykinin. After removal of the peptide, from either the mucosal or the serosal side of the bladder, the effects of the neurohypophysial hormone were completely restored.

Oxytocin normally increases the permeability of the toad bladder to water. Table 3 shows that when the serosal side of the organ was exposed to bradykinin (9.5×10^{-7} M), the action of oxytocin was inhibited in a manner similar to that observed with vasopression.

Dose/response relationships to vasopression and oxytocin were affected by bradykinin in a very similar way. The inhibitory effect of 9.5×10^{-7} M bradykinin on the action of vasopressin (or oxytocin) could be readily overcome by increasing the concentration of the pituitary hormone so that

Table 1. *Hydro-osmotic response of the toad bladder to vasopressin in the absence and in the presence of bradykinin* [a]

Bradykinin	Osmotic water flow (mg/min)			
	Vasopressin concn. (M)			
	2.6×10^{-9}	1.3×10^{-8}	6.5×10^{-8}	3.3×10^{-7}
None	8.0	14.0	27.5	30.0
	9.0	13.5	27.0	30.0
	8.5	15.0	25.0	38.0
	8.0	20.0	25.0	36.0
	9.0	20.0	25.5	36.0
	(8.5)[b]	(16.5)	(26.0)	(34.0)
9.5×10^{-7} M	5.5	7.0	16.0	24.0
	5.0	8.0	20.0	22.0
	4.5	10.0	17.0	31.0
	4.0	9.0	19.0	27.0
	4.0	11.0	18.0	26.0
	(4.5)	(9.0)	(18.0)	(26.0)

[a] Both vasopressin and bradykinin were added to the serosal bath in paired experiments.

[b] Numbers in parentheses represent means.

Table 2. *Hydro-osmotic response of the toad bladder to vasopressin in the absence and in the presence of* 9.5×10^{-7} M *bradykinin in paired experiments* [a]

Bradykinin added to	Osmotic water flow (mg/min)							
	Vasopressin concn. (M)							
	2.6×10^{-9}		1.3×10^{-8}		6.5×10^{-8}		3.3×10^{-7}	
	$\bar{S}$ [b]	$\bar{C}$ [c]	$\bar{S}$	$\bar{C}$	$\bar{S}$	$\bar{C}$	$\bar{S}$	$\bar{C}$
Serosal solution	12.0	6.0	18.0	12.0	25.0	20.0	30.0	20.0
	9.0	2.5	15.0	10.0	30.0	20.0	39.0	35.0
	11.0	2.0	20.0	12.0	26.0	20.0	36.0	27.0
	(10.5)[d]	(3.5)	(17.5)	(11.5)	(27.0)	(20.0)	(35.0)	(27.0)
Mucosal solution	10.0	4.0	16.0	10.0	25.0	16.0	30.0	20.0
	9.0	2.0	18.0	10.0	26.0	19.0	36.0	33.0
	9.0	3.0	18.0	12.0	28.0	17.0	32.0	31.0
	(9.5)	(3.0)	(17.5)	(10.5)	(26.0)	(17.5)	(33.0)	(28.0)

[a] Vasopressin was added to the serosal bath.
[b] $\bar{S}$ = in the absence of bradykinin.
[c] $\bar{C}$ = in the presence of bradykinin.
[d] Numbers in parentheses represent means.

Table 3. *Hydro-osmotic response of the toad bladder to oxytocin in the absence and in the presence of bradykinin* [a]

Bradykinin	Osmotic water flow (mg/min)			
	Oxytocin concn. (M)			
	2×10^{-8}	1×10^{-8}	5×10^{-8}	2.5×10^{-7}
None	5.0	14.0	23.5	24.0
	9.5	14.0	22.5	21.0
	9.5	19.0	23.0	27.5
	8.0	17.0	23.0	36.0
	7.0	14.0	23.5	30.0
	(8.5)[b]	(15.5)	(23.0)	(28.0)
9.5×10^{-7} M	1.0	4.5	14.0	23.0
	2.5	9.5	15.0	24.0
	2.0	9.5	20.0	20.0
	3.0	9.0	16.5	25.0
	1.5	8.5	13.5	28.0
	(2.0)	(8.0)	(16.0)	(24.0)

[a] Both oxytocin and bradykinin were added to the serosal bath in paired experiments.
[b] Numbers in parentheses represent means.

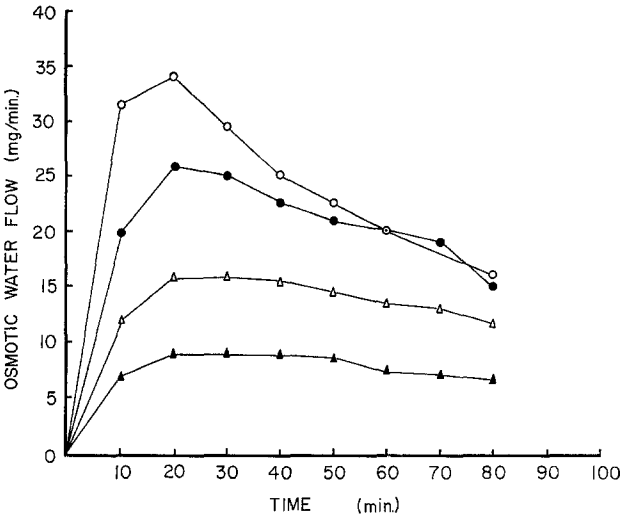

Fig. 1. Effects of different concentrations of vasopressin on the osmotic water movemen across the wall of the isolated toad bladder as a function of time. The peptide was adde to the serosal Ringer's solution and the concentrations used were as follows: 2.6×10^{-9} M (▲); 1.3×10^{-8} M (△); 6.5×10^{-8} M (●); and 3.3×10^{-7} M (○). Each point represents th mean of five determinations

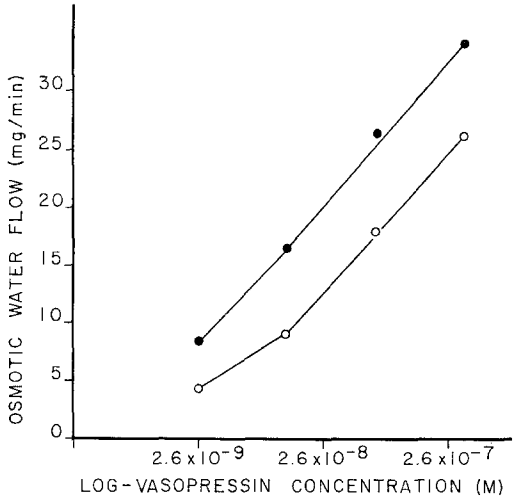

Fig. 2. Relationship of osmotic water transfer across the bladder to the concentratio of vasopressin present, measured in the absence (●) and in the presence (○) of 9.5×10^{-7} M bradykinin. Each point represents the mean of five determinations

maximal or near-maximal responses were still obtained despite the presenc of the inhibitor. This finding leaves open the possibility of the antagonism being competitive in the presence of such a concentration of bradykinin As shown in Figs. 2 and 3, the shape of the log-dose/response curves fo

vasopressin and oxytocin both in the absence and presence of bradykinin was the same. However, the addition of the kinin caused a parallel shift of the curve so that the dose of vasopressin (or oxytocin) required to overcome the effects of 9.5×10^{-7} M bradykinin was about four (to five) times the control dose. The character of the curves indicated that the differences in sensitivity may have been caused by an altered affinity of the hormones for their receptor sites (Ariëns & Groot, 1954).

Kallidin. The increased water permeability of the bladder in the presence of vasopressin was reduced when kallidin (8.5×10^{-7} M) was added to the serosal solution together with the pituitary hormone (Table 4). The inhibition was again reversible, the effect of vasopressin being completely restored after washing the preparation. It is also apparent from Table 4 and Fig. 4 that the inhibition of vasopressin by kallidin was very similar to that observed with bradykinin. Kallidin affected dose/response relationships to vasopressin in a manner qualitatively similar to that of bradykinin. The antagonism again apparently altered the affinity of the neurohypophysial hormone for its receptors, causing a parallel shift of the log-dose/response curve to the right along the axis of the abscissae. The dose of vasopressin required to overcome the effects of 8.5×10^{-7} M kallidin was about six times the control dose.

Table 4. *Hydro-osmotic response of the toad bladder to vasopressin in the absence and in the presence of kallidin*[a]

Kallidin	Osmotic water flow (mg/min)				
	Vasopressin concn. (M)				
	2.6×10^{-9}	1.3×10^{-8}	1.3×10^{-7}	3.3×10^{-7}	5.2×10^{-7}
None	10.0	13.0	35.5	39.0	32.0
	7.0	17.5	29.5	38.0	34.5
	9.0	21.5	34.5	38.5	47.5
	7.5	20.0	29.5	38.5	32.0
	11.0	13.0	36.0	42.5	34.5
	(9.0)[b]	(17.0)	(33.0)	(39.0)	(36.0)
8.5×10^{-7} M	3.0	5.0	25.5	28.0	35.5
	1.0	3.0	21.0	24.5	36.5
	1.5	4.0	21.5	23.5	44.0
	1.0	8.0	19.0	32.0	30.5
	5.5	7.0	18.0	28.0	38.5
	(2.5)	(5.5)	(21.0)	(27.0)	(37.0)

[a] Both vasopressin and kallidin were added to the serosal bath in paired experiments.
[b] Numbers in parentheses represent means.

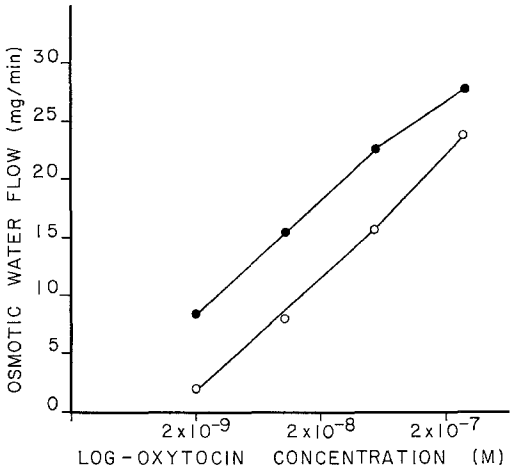

Fig. 3. Hydro-osmotic effects of oxytocin on the toad bladder in the absence (•) and i the presence (◦) of 9.5×10^{-7} M bradykinin. Each point is the mean of five values

Eledoisin. Exposure of the serosal side of the bladder to 8.5×10^{-7} M eledoisin, in the presence of increasing concentrations of vasopressin, resulte in a pronounced inhibition of the hydro-osmotic effect of the pituitar hormone (Table 5, Fig. 5), necessitating a dose of vasopressin approximatel

Table 5. *Hydro-osmotic response of the toad bladder to vasopression in the absence and i the presence of eledoisin* [a]

Eledoisin	Osmotic water flow (mg/min)				
	Vasopressin concn. (M)				
	2.6×10^{-9}	1.3×10^{-8}	2.6×10^{-8}	1.3×10^{-7}	2.6×10^{-7}
None	17.0	23.0	28.5	35.0	49.0
	16.0	25.0	30.0	45.0	41.0
	12.5	23.0	26.5	42.0	38.0
	10.0	18.0	27.5	35.0	48.0
	12.0	17.0	25.0	35.0	35.0
	(13.0)[b]	(21.0)	(27.5)	(38.0)	(42.0)
8.5×10^{-7} M	11.0	13.0	18.5	26.0	44.0
	10.0	18.0	20.5	32.0	23.0
	7.0	17.0	18.5	23.0	28.0
	5.0	8.0	18.5	28.0	40.0
	9.0	10.0	17.0	28.0	22.0
	(8.0)	(13.0)	(18.5)	(27.5)	(31.5)

[a] Both vasopressin and eledoisin were added to the serosal bath in paired experiments.

[b] Numbers in parentheses represent means.

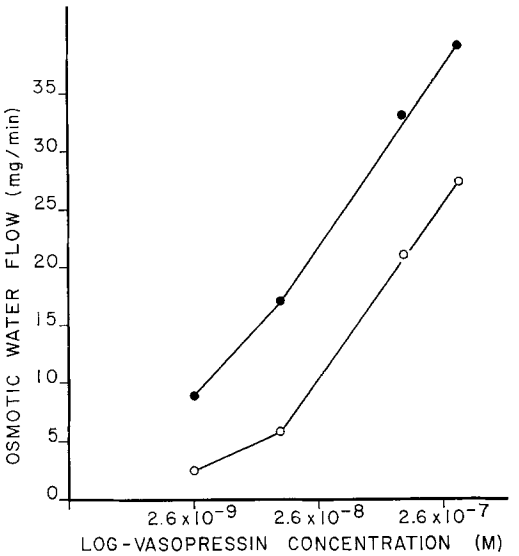

Fig. 4. Hydro-osmotic response of the toad bladder to vasopressin in the absence (○) and in the presence (•) of 8.5×10^{-7} M kallidin. Each point is the mean of five values

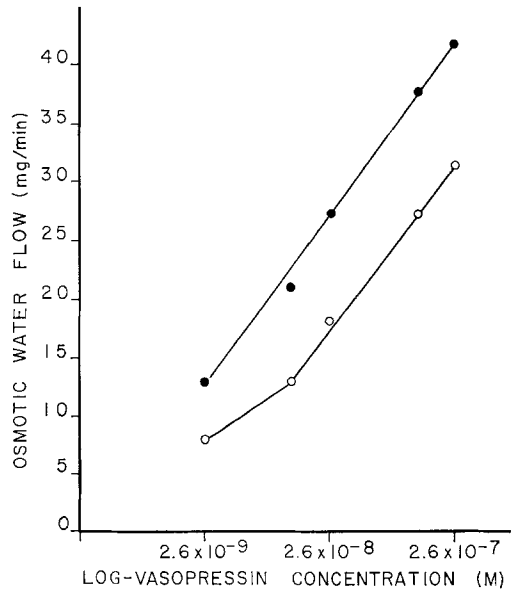

Fig. 5. Log-dose/response relationship for the effects of vasopressin on the permeability to water of the toad bladder in the absence (•) and in the presence (○) of 8.5×10^{-7} M eledoisin. Each point is the mean of five values

five times the control in order to overcome the inhibition of 8.5×10^{-7} M eledoisin. Oxytocin was also inhibited by eledoisin in a manner qualitatively similar to that observed with vasopressin. Both neurohypophysial hormones

Table 6. *Hydro-osmotic response of the toad bladder to vasopressin in the absence an in the presence of physalaemin*[a]

Physalaemin	Osmotic water flow (mg/min)			
	Vasopressin concn. (M)			
	2.6×10^{-9}	1.3×10^{-8}	6.5×10^{-8}	3.3×10^{-7}
None	7.0	20.5	30.0	34.5
	7.5	20.0	38.0	34.0
	8.0	21.0	28.0	38.0
	9.0	23.0	32.0	40.0
	8.0	19.0	24.0	50.0
	(8.0)[b]	(20.5)	(30.5)	(39.5)
8.0×10^{-7} M	2.5	13.0	25.0	27.0
	2.5	11.0	27.0	27.0
	2.5	10.5	20.0	37.0
	3.0	14.0	22.0	28.0
	2.5	8.5	15.0	36.0
	(2.6)	(11.5)	(22.0)	(31.0)

[a] Both vasopressin and physalaemin were added to the serosal bath in paired experiments.

[b] Numbers in parentheses represent means.

were reversibly inhibited by eledoisin, the normal responsiveness of the bladder tissue being restored after the preparation was washed.

Physalaemin. This peptide also acted as an inhibitor of vasopressin and oxytocin when applied to the serosal side of the bladder, the effects being reversible. Table 6 and Figs. 6 and 7 show that 8.0×10^{-7} M physalaemin affected dose/response relationships to neurohypophysial hormones in a way that resembles eledoisin, apparently affecting the affinity of vasopressin (or oxytocin) to receptor sites. The presence of physalaemin (8.0×10^{-7} M) required the utilization of a dose of vasopressin that was approximately four times greater than the control one. On the other hand, the dose of oxytocin required to overcome the effects of 8.0×10^{-7} M physalaemin was about 10 times the control dose.

Theophylline. The xanthines have also been shown to increase the permeability of the toad bladder to water (Orloff & Handler, 1962). Four different concentrations of theophylline were utilized in paired experiments. Each dose, with and without bradykinin, was assayed in five single pairs of bladders. The average hydro-osmotic responses to the various doses of

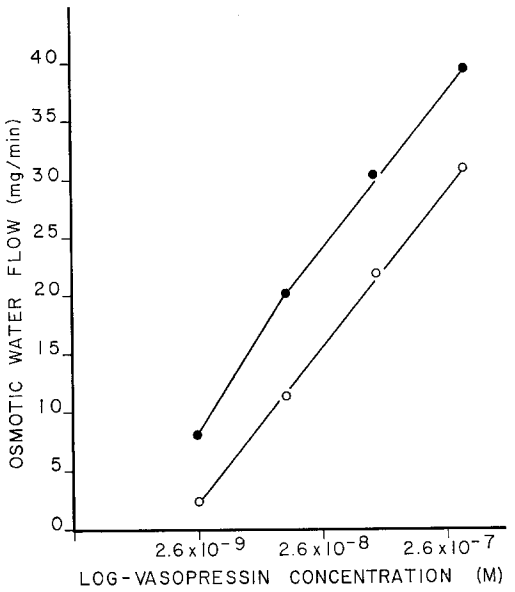

Fig. 6. Hydro-osmotic effects of vasopressin on the toad bladder in the absence (•) and in the presence (◦) of 8.0×10^{-7} M physalaemin. Each point is the mean of five values

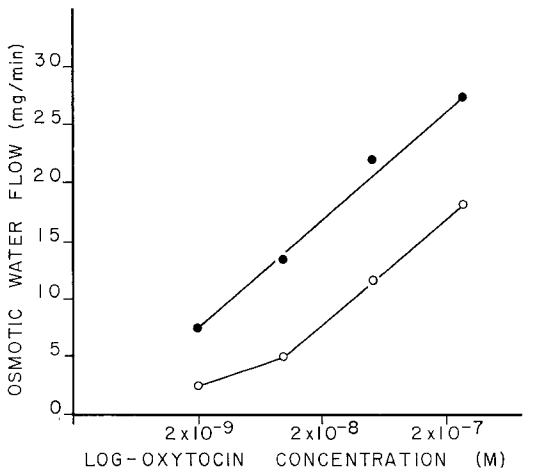

Fig. 7. Hydro-osmotic response of the toad bladder to oxytocin in the absence (◦) and in the presence (•) of 8.0×10^{-7} M physalaemin. Each point is the mean of three determinations

theophylline were as follows: 5×10^{-6} M $= 3.0 \pm 0.5$ mg/min; 10^{-5} M $= 12.0 \pm 2.0$ mg/min; 2×10^{-5} M $= 20.5 \pm 2.8$ mg/min; and 4×10^{-5} M $= 29.0 \pm 2.9$ mg/min. These responses were not affected by 9.5×10^{-7} M bradykinin when both drugs were applied to the serosal side of the bladder. The average

osmotic water transfer across the bladder in response to those same dos of theophylline in the presence of bradykinin was: 5×10^{-6} M = 2.8 0.5 mg/min; 10^{-5} M = 14.0 ± 3.7 mg/min; 2×10^{-5} M = 22.0 ± 3.3 mg/mi and 4×10^{-5} M = 29.0 ± 2.3 mg/min.

Discussion

The present studies show that kinins are potent inhibitors of neur hypophysial hormones in the isolated toad bladder. Interpretation of t log-dose/response curves would indicate that bradykinin, kallidin, eledoisi and physalaemin might be acting as competitive antagonists to vasopressi and oxytocin: although lacking intrinsic activity, these kinin hormon exhibited affinity for receptor sites that are also common to the neur hypophysial peptides, causing a parallel displacement of the log-dos response curves for the pituitary hormones along the axis of the abscissa This is in sharp contrast to what was observed with the octapeptide angi tensin-II, which at a dosage of 9.5×10^{-7} M did not affect the respons of the bladder to 2.6×10^{-9} M vasopressin.

According to the drug-receptor theory, the neurohypophysial hormon must first attach to, or at least interact with, a receptor site in a specif manner before it can initiate its biological effect. A high degree of molecul complementarity between hormones and receptor sites is to be expecte (Rasmussen, Schwartz, Young & Marc-Aurele, 1963) suggesting sever possible points of interaction between kinins and pituitary peptides wit bladder receptor sites. At the moment, attempts to clarify the issue will b delayed for two main reasons: (1) because of the marked structural di ferences among the kinins and between them and the neurohypophysi peptides; and (2) because of the fact that angiotensin-II was found to b inactive against vasopressin while the other kinins were observed to b inhibitors in spite of having as unspecific a structure as angiotensin. Furth work utilizing different analogues of both the neurohypophysial peptid and the kinins will show the importance of the various positions in th molecule of each peptide to the process of attachment to receptor sit (therefore, the process of competition between the two classes of peptides).

It has been observed that toads are generally more sensitive to vas pressin than to oxytocin as far as water permeability is concerned (Ewe 1951, 1952; Sawyer & Sawyer, 1952; Heller, 1963), whereas the revers seems to be true of frogs (Boyd & Brown, 1938; Ewer, 1951, 1952; Helle 1963). The present work is consonant with the former observation: vas pressin showed greater activity than oxytocin in the toad bladder. Moreove

it was observed that bradykinin as well as physalaemin inhibited oxytocin more than vasopressin, apparently acting as competitive antagonists whichever the hormone utilized. These findings, taken together, support the view that either vasopressin and oxytocin have different affinity constants for the same receptor sites, or there is not identity of receptors for the neurohypophysial peptides in the urinary bladder of the toad, suggesting a different population of receptor sites for vasopressin and oxytocin.

Orloff and Handler (1962) have advanced the hypothesis that the interaction of neurohypophysial hormone and receptor site leads to an increase in the rate of production of cyclic 3′,5′-AMP through the activation of the enzyme adenyl cyclase. The cyclic-AMP would then, by some as-yet-unknown mechanism, increase the permeability of the bladder wall. If this is true, the kinins could also act directly but reversibly on either adenyl cyclase, (and therefore on the generation of cyclic-AMP) or on the interaction between cyclic-AMP and the effector site. According to the same hypothesis, a similar hormone-like response is elicited by theophylline which does so by inhibiting the inactivation of endogenously formed cyclic-AMP. If this is correct, any drug that antagonizes cyclic-AMP *per se* would also preclude the effect of theophylline. Our finding of the absence of inhibition of theophylline by bradykinin suggests that this drug would not antagonize cyclic-AMP either, and points to a rather specific antagonism between bradykinin and the neurohypophysial hormones.

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