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EFFECT OF BRADYKININ ON TRANSEPITHELIAL TRANSFER OF SODIUM AND WATER *IN VITRO*

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SUMMARY

1. Mucosal sodium and water transfer were measured in everted sacs of rat jejunum.

2. Bradykinin ($7{\cdot}86 \times 10^{-12}$ M), when present in both mucosal and serosal solutions, produced a biphasic effect on mucosal sodium and water transfer. When basal transfer was low a stimulation was observed whereas an inhibition of transfer was observed when basal transfer was high. Bradykinin at concentrations of $7{\cdot}86 \times 10^{-11}$ and $7{\cdot}86 \times 10^{-13}$ M produced qualitatively similar effects.

3. Inhibition of transfer was observed whether bradykinin was present in the mucosal, serosal or both solutions. Stimulation of transfer was observed only when bradykinin was present in the serosal solution.

4. Theophylline (1 mM), alone, inhibited water transfer at high and intermediate levels of basal transfer, and significantly potentiated the inhibitory effect of bradykinin ($7{\cdot}86 \times 10^{-12}$ M) on water transfer at intermediate levels of control transfer.

5. Cyclic AMP (1 mM) inhibited water transfer when basal transfer was high. Dibutyryl cyclic AMP (1 mM) inhibited water transfer at all levels of basal transfer. Dibutyryl cyclic AMP (1 mM) and bradykinin ($7{\cdot}86 \times 10^{-12}$ M) together produced a significantly greater inhibition of water transfer than either agent alone, at intermediate basal transfer.

6. It was observed that the action of bradykinin upon sodium and water transfer consists of two different and opposing effects. It is possible that the inhibitory effect of bradykinin upon water transfer is related to increased cyclic AMP activity.

INTRODUCTION

Bradykinin has been reported to affect sodium and water movements in kidney and toad bladder. An infusion of bradykinin into the renal artery produced a diuresis and a natriuresis (Barraclough & Mills, 1965), which

could be due either to haemodynamic changes within the kidney (Earley & Friedler, 1966; Willis, Ludens, Hook & Williamson, 1969) or to a direct action on tubular transport of sodium and water (Alzamora & Capelo, 1973). However, in toad bladder, bradykinin brings about changes in sodium movement by affecting the natriferic action of vasopressin rather than by a direct action on sodium transporting mechanisms (Furtado & Machado, 1966). The present study was undertaken to investigate if bradykinin affected intestinal sodium and water transfer using an *in vitro* method to determine if any such effect was a result of a direct action on sodium transporting mechanisms.

METHODS

Male Wistar rats, 100–200 g body weight, were starved for 18 hr before experimentation. Rats were killed by a blow on the head and the whole of the small intestine was rinsed through with sodium chloride solution (0·154 M). The intestine was everted according to the technique of Wilson & Wiseman (1954), and then divided into five equal segments (Barry, Matthews & Smyth, 1961). The jejunal segment (sac II) was subdivided into two equal sacs so that each experimental animal provided two adjacent sacs. One of these sacs served as a control and was filled with Krebs bicarbonate solution containing 27·75 mM glucose while the other sac was filled with the same solution to which had been added the substance under investigation. The proximal sac made from the jejunal segment of each animal was used alternately as a control or an experimental sac so that any effect that might have been due to either the delay in using the sacs, or to differences in transport characteristics along the intestine, were minimized.

Each sac was weighed, then filled with the appropriate solution and tied off. The sac was reweighed and incubated in an Ehrlenmeyer flask containing 15 ml. Krebs bicarbonate solution which had been gassed with 95% O_2 + 5% CO_2. The composition of the Krebs bicarbonate solution was the same in mucosal and serosal solutions and contained (mM): NaCl 118, KCl 4·7, $MgSO_4.7H_2O$ 1·18, KH_2PO_4 1·17, $NaHCO_3$ 25, $CaCl_2 2H_2O$ 2·5, glucose 27·75, pH 7·4. The sacs were incubated at 37 °C for an hour in a shaking water-bath at 40 oscillations/min. At the end of the incubation period the sacs were blotted dry and reweighed. Samples of the mucosal solutions were estimated for sodium using a Pye Unicam SP90 series 2 Atomic Absorption spectrophotometer.

Results were expressed as mucosal transfer, that is the amount of sodium or water leaving the mucosal solution during the incubation period as described by Parsons, Smyth & Taylor (1958) and discussed by Smyth (1963). The mucosal water transfer was calculated from the weight changes in the sacs after incubation and the mucosal sodium transfer was calculated from a knowledge of the quantities of sodium present initially and finally in the mucosal solution. Water transfer was expressed as ml./g initial wet weight of jejunal sac.hr and sodium transfer as μequiv/g initial wet weight of jejunal sac.hr.

In the Results section a negative (−) sign is used to denote an inhibition of control transfer while a positive (+) sign is used to denote a stimulation of control transfer. The values quoted in the text refer to the % change from the control transfer.

Drugs

Bradykinin triacetate (Sigma Chemical Co.) was dissolved in distilled water and stored at 0° C in sealed ampoules at a concentration of $7{\cdot}86 \times 10^{-8}$ M. It was diluted to the required concentration immediately before use.

Cyclic AMP and dibutyryl cyclic AMP (Sigma Chemical Co.) were stored in a desiccator at 4° C and solutions were prepared immediately before use.

Theophylline (Sigma Chemical Co.) was stored at room temperature and solutions were prepared immediately before use.

In all experiments, unless otherwise specified, bradykinin was present in both mucosal and serosal solutions. Theophylline was always present in both mucosal and serosal solutions and both cyclic AMP and dibutyryl cyclic AMP were present only in the serosal solution.

Statistics

The results were expressed as mean ± S.E. of mean and were analysed statistically using the Student's *t* test.

RESULTS

(1) *Effect of bradykinin on sodium and water transfer*

Initially it appeared that the effect of bradykinin on sodium and water transfer was variable but upon closer scrutiny of the results it became apparent that the effect produced by bradykinin was dependent upon the level of sodium and water transfer in the paired control sac which had been incubated in Krebs bicarbonate solution alone (Fig. 1). Thus a stimulation of water transfer was observed in all sacs when the control transfer in the paired sac was low, i.e. less than 0·6 ml./g wet wt. hr. When transfer levels were intermediate, i.e. between 0·6 and 1·0 ml./g wet wt. hr bradykinin produced either a stimulation, an inhibition or no effect but when level of transfer in the control sac was high, i.e. greater than 1·0 ml./g wet wt. hr, bradykinin produced an inhibition of transfer in all sacs. The effects of bradykinin, $7{\cdot}86 \times 10^{-12}$ M, on water transfer are shown in Fig. 1 and were associated with corresponding changes in sodium transfer as reported by Curran & Solomon (1957). The results in Table 1 show that a similar pattern of effects on water transfer were obtained when bradykinin at $7{\cdot}86 \times 10^{-11}$ or $7{\cdot}86 \times 10^{-13}$ M was present in both mucosal and serosal solutions. At low levels of control transfer bradykinin $7{\cdot}86 \times 10^{-11}$, $7{\cdot}86 \times 10^{-12}$ and $7{\cdot}86 \times 10^{-13}$ M significantly stimulated water transfer by +67·2, +37·8 and +27·9 % respectively. Water transfer was unaffected by bradykinin at any concentration when control transfer was intermediate but at high control transfer bradykinin at concentrations of $7{\cdot}86 \times 10^{-11}$, $7{\cdot}86 \times 10^{-12}$ and $7{\cdot}86 \times 10^{-13}$ M significantly inhibited water transfer by −33·4, −22·4 and −19·9 % respectively. Again these effects were associated with changes in sodium transfer although these data are not included in this or subsequent tables.

Mean mucosal water transfer

Control	BK	Control	BK	Control	BK
0·519 ± 0·017	0·715 ± 0·047	0·780 ± 0·035	0·758 ± 0·041	1·139 ± 0·026	0·883 ± 0·053
$n = 17; P = < 0·001$		$n = 31; P = > 0·60$		$n = 17; P = < 0·001$	

Mean mucosal sodium transfer

46·3 ± 5·4	68·9 ± 7·7	87·1 ± 5·8	85·9 ± 5·7	159·4 ± 5·3	127·7 ± 5·9
$n = 12; P = < 0·02$		$n = 22; P = > 0·80$		$n = 15; P = < 0·01$	

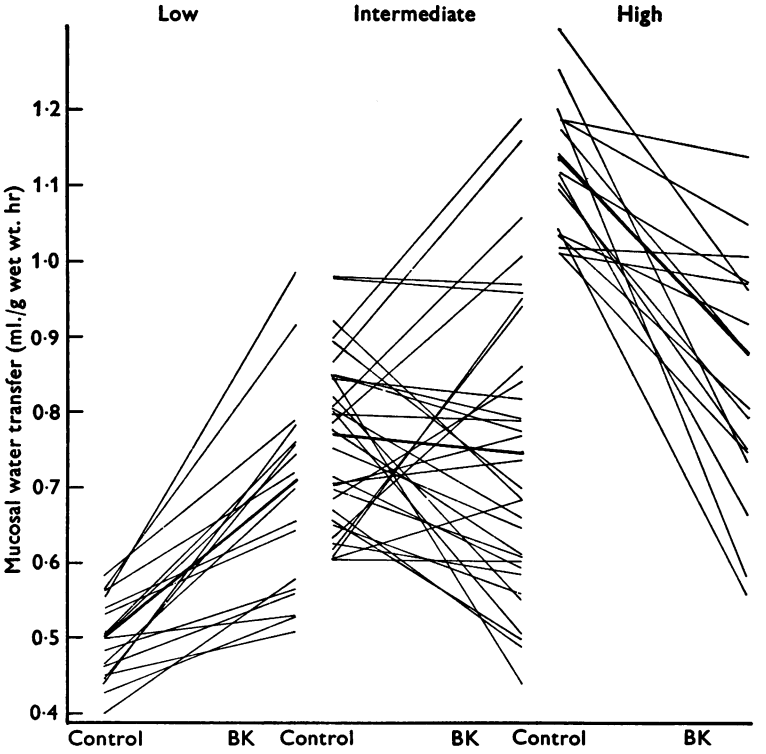

Fig. 1. Effect of bradykinin (BK), $7·86 \times 10^{-12}$ M, in mucosal and serosal solutions on sodium and water transfer expressed as ml. or μequiv/g wet wt. of jejunal sac.hr. The degrees of significance are expressed as follows: * = 0·05; ** = 0·02; *** = 0·01; **** = 0·001; n.s. = not significant. The number of observations are given in parentheses. Means are represented by heavy line.

Bradykinin at $7{\cdot}86 \times 10^{-12}$ M produced a consistent qualitative and quantitative effect in subsequent experiments reported here.

Values of water transfer in control sacs were compared with the paired experimental sacs which had been incubated with bradykinin, $7{\cdot}86 \times 10^{-12}$ M, in either the mucosal or serosal solution or in both solutions (Table 1). Water transfer was significantly inhibited by bradykinin at high control transfer when it was present in either the mucosal or serosal solutions, or both, but at intermediate levels of control water transfer bradykinin had no significant effect. A significant stimulation of water transfer at low levels of control transfer was obtained when bradykinin was present in both solutions, or in the serosal solution only, but no effect was present when bradykinin was present only in the mucosal solution.

TABLE 1. *A*, the effect of bradykinin, $7{\cdot}86 \times 10^{-11}$ and $7{\cdot}86 \times 10^{-13}$ M on water transfer in rat jejunal sacs. *B*, the effect of bradykinin, $7{\cdot}86 \times 10^{-12}$ M, in either mucosal or serosal solution in rat jejunal sacs. Bradykinin, unless otherwise stated, is present in both mucosal and serosal solutions. The degree of significance for each set of results is expressed as follows: * = < 0·05; ** = < 0·02; *** = < 0·01; **** = < 0·001; n.s. = not significant. The number of observations in each case is given in parentheses

Group	Level of mucosal water transfer (ml./g wet wt. hr) Low	Intermediate	High
A			
Control	0·528 ± 0·033	0·728 ± 0·037	1·178 ± 0·045
Bradykinin ($7{\cdot}86 \times 10^{-11}$ M)	0·884 ± 0·064*** (4)	0·794 ± 0·051 n.s. (18)	0·789 ± 0·046**** (9)
Control	0·468 ± 0·019	0·761 ± 0·027	1·081 ± 0·054
Bradykinin ($7{\cdot}86 \times 10^{-13}$ M)	0·596 ± 0·053** (14)	0·738 ± 0·043 n.s. (28)	0·865 ± 0·083* (6)
B			
Control	0·451 ± 0·021	0·722 ± 0·062	1·145 ± 0·043
Bradykinin	0·612 ± 0·033**** (14)	0·668 ± 0·062 n.s. (6)	0·927 ± 0·062*** (11)
Control	0·557 ± 0·027	0·742 ± 0·026	1·179 ± 0·043
Bradykinin (mucosal)	0·579 ± 0·042 n.s. (6)	0·725 ± 0·043 n.s. (18)	0·814 ± 0·081**** (9)
Control	0·527 ± 0·036	0·786 ± 0·034	1·083 ± 0·044
Bradykinin (serosal)	0·750 ± 0·072*** (7)	0·761 ± 0·043 n.s. (9)	0·841 ± 0·062*** (4)

(2) *Effect of theophylline (1 mM) and bradykinin ($7{\cdot}86 \times 10^{-12}$ M) on water transfer*

At low levels of control transfer the stimulation produced by bradykinin, + 33·68 ± 12·79 %, was converted to an inhibition, − 14·38 ± 1·68 %, by a concentration of theophylline which alone was without effect (Table 2).

Bradykinin and theophylline produced a significant inhibition of water transfer, $-33{\cdot}45 \pm 3{\cdot}17$ %, at intermediate levels of control transfer which was significantly greater ($P < 0{\cdot}01$) than that produced by theophylline alone, $-17{\cdot}81 \pm 4{\cdot}30$ %. At high levels of control transfer bradykinin and theophylline produced a significant inhibition of water transfer, $-45{\cdot}95 \pm 5{\cdot}32$ %, which was significantly greater than that produced by bradykinin, $-14{\cdot}48 \pm 3{\cdot}63$ %, but not significantly different from that produced by theophylline, $-38{\cdot}64 \pm 4{\cdot}43$ %.

TABLE 2. The effect of theophylline (1 mM) and bradykinin, $7{\cdot}86 \times 10^{-12}$ M, on water transfer in rat jejunal sacs. Both theophylline and bradykinin were present in both mucosal and serosal solutions. Degrees of significance are expressed in a manner similar to that used in Table 1

Group	Level of mucosal water transfer (ml./g wet wt. hr)		
	Low	Intermediate	High
Control	$0{\cdot}491 \pm 0{\cdot}033$	$0{\cdot}725 \pm 0{\cdot}025$	$1{\cdot}152 \pm 0{\cdot}046$
Bradykinin	$0{\cdot}653 \pm 0{\cdot}063$* (6)	$0{\cdot}768 \pm 0{\cdot}051$ n.s (10)	$0{\cdot}978 \pm 0{\cdot}019$*** (5)
Control	$0{\cdot}471 \pm 0{\cdot}034$	$0{\cdot}797 \pm 0{\cdot}032$	$1{\cdot}210 \pm 0{\cdot}081$
Theophylline	$0{\cdot}468 \pm 0{\cdot}029$ n.s. (6)	$0{\cdot}648 \pm 0{\cdot}036$ n.s. (10)	$0{\cdot}728 \pm 0{\cdot}045$**** (6)
Control	$0{\cdot}517 \pm 0{\cdot}033$	$0{\cdot}823 \pm 0{\cdot}023$	$1{\cdot}148 \pm 0{\cdot}061$
Theophylline and bradykinin	$0{\cdot}442 \pm 0{\cdot}029$* (5)	$0{\cdot}545 \pm 0{\cdot}032$**** (10)	$0{\cdot}612 \pm 0{\cdot}037$**** (5)

(3) *Effect of cyclic AMP (1 mM), dibutyryl cyclic AMP (1 mM), and bradykinin ($7{\cdot}86 \times 10^{-12}$ M) on water transfer*

Cyclic AMP inhibited water transfer significantly at high control transfer levels (Table 3) but no significant effect was seen at intermediate transfer levels.

The effect of dibutyryl cyclic AMP, alone or in combination with bradykinin was investigated (Table 3). Dibutyryl cyclic AMP inhibited water transfer significantly when the control transfer was low, $-19{\cdot}65 \pm 2{\cdot}69$ %; intermediate, $-13{\cdot}49 \pm 5{\cdot}19$ %; or high, $-13{\cdot}62 \pm 2{\cdot}73$ %. At low levels of control transfer bradykinin and dibutyryl cyclic AMP together stimulated transfer by a smaller amount, $+15{\cdot}31 \pm 2{\cdot}47$ %, than that produced by bradykinin alone, $+28{\cdot}37 \pm 8{\cdot}45$ %, and at intermediate levels of control transfer produced an inhibition of water transfer, $-28{\cdot}59 \pm 3{\cdot}05$ %, which was significantly greater ($P < 0{\cdot}01$) than that produced by dibutyryl cyclic AMP alone, $-13{\cdot}49 \pm 5{\cdot}19$ %. When control transfer was high bradykinin and dibutyryl cyclic AMP produced an inhibition of water transfer, $-33{\cdot}56 \pm 2{\cdot}99$ %, which was greater than that produced by either

TABLE 3. The effect of cyclic AMP (1 mM), dibutyryl cyclic AMP (1 mM), and bradykinin ($7{\cdot}86 \times 10^{-12}$ M) on water transfer in rat jejunal sacs. Bradykinin was present in both mucosal and serosal solutions, while cyclic AMP and dibutyryl cyclic AMP were present only in the serosal solution. Degrees of significance are expressed in a manner similar to that used in Table 1

	Level of mucosal water transfer (ml./g wet wt. hr)		
Group	Low	Intermediate	High
Control	0·583 ± 0·026	0·819 ± 0·041	1·134 ± 0·052
Cyclic AMP	0·564 ± 0·103 (2)	0·826 ± 0·046 n.s. (7)	0·868 ± 0·043*** (6)
Control	0·500 ± 0·033	0·704 ± 0·023	1·123 ± 0·034
Bradykinin	0·636 ± 0·051* (8)	0·699 ± 0·056 n.s. (12)	0·929 ± 0·035*** (6)
Control	0·548 ± 0·021	0·801 ± 0·033	1·094 ± 0·046
Dibutyryl cyclic AMP	0·438 ± 0·026*** (5)	0·675 ± 0·023*** (16)	0·942 ± 0·022*** (6)
Control	0·525 ± 0·026	0·811 ± 0·023	1·147 ± 0·043
Dibutyryl cyclic AMP and bradykinin	0·606 ± 0·023* (4)	0·579 ± 0·034**** (14)	0·758 ± 0·036*** (7)

bradykinin, $-17{\cdot}02 \pm 3{\cdot}03$ %, or dibutyryl cyclic AMP, $-13{\cdot}62 \pm 2{\cdot}73$ %, separately.

DISCUSSION

Bradykinin was observed to have two different effects on the transfer of sodium and water which appeared to be dependent on the control level of transfer. The effects of bradykinin on water transfer were associated with corresponding changes in sodium transfer.

Dennhardt & Haberich (1973) reported that kallikrein could have both stimulatory and inhibitory effects on the transport of sodium and water by rat jejunum and colon. It is possible to interpret their results to support the idea that the observation of an inhibition or a stimulation of transfer by kallikrein appeared to be dependent on the basal level of transfer in the tissue, thus suggesting a similarity between our findings and theirs. Bradykinin has been reported to produce two opposite effects in other systems such as intestinal smooth muscle (Elliott, Horton & Lewis, 1960) and taenia coli (Aarsen & van Caspel-de Bruyn, 1970). The concept, therefore, that bradykinin may have two opposing effects, and that either of these effects may be dominant depending on the prevailing conditions, is not new.

The stimulatory action of bradykinin exhibited a clear dose response effect so that a greater stimulatory effect was seen with higher concentrations. However, this relationship was less clear when the inhibitory action

of bradykinin was considered. Concentrations of $7{\cdot}86 \times 10^{-12}$ and $7{\cdot}86 \times 10^{-13}$ M produced a similar inhibition of transfer but $7{\cdot}86 \times 10^{-11}$ M produced a greater degree of inhibition. It may be that the concentrations of bradykinin chosen are towards the lower end of the dose response curve for the inhibitory effect of bradykinin since the dose–response curves for the stimulatory and inhibitory effects of bradykinin may not be the same.

It seems likely that the mechanisms responsible for the inhibitory and stimulatory effects of bradykinin on transfer may be different since the inhibitory effect is observed when bradykinin is present in either the serosal or mucosal solution, whereas for the stimulatory effect bradykinin must be present in the serosal solution. Although the present work gives no indication of the nature of the stimulatory effect, findings from experiments using theophylline and cyclic AMP do suggest a possible explanation of some of the mechanisms involved in the inhibitory action of bradykinin on mucosal sodium and water transfer.

Theophylline inhibited transfer of water at high and intermediate levels of control transfer and this is in agreement with other reports in the literature (Field, Fromm & Silen, 1969; Hornych, Meyer & Milliez, 1973). It was observed that bradykinin and theophylline together always produced an inhibition of water transfer regardless of the level of control transfer. At low levels of control water transfer the stimulatory effect of bradykinin was converted to one of inhibition by theophylline at a concentration which alone was without effect. Bradykinin had no effect on water transfer at intermediate levels of control transfer but in combination with theophylline produced an inhibition of water transfer which was significantly greater than that produced by theophylline alone. At high levels of control transfer bradykinin and theophylline produced an inhibition of water transfer which was slightly less than the arithmetic sum of the degrees of inhibition produced by bradykinin and theophylline separately, possibly suggesting that a maximum degree of inhibition had been reached at this level of control transfer. Thus the inhibitory effect of bradykinin was potentiated by theophylline which has been reported to prevent break-down of cyclic AMP by inhibition of the enzyme phosphodiesterase (Butcher & Sutherland, 1962). The observations with bradykinin and theophylline raise the possibility that cyclic AMP may be involved in the inhibitory action of bradykinin on water transfer.

In the present study cyclic AMP inhibited water transfer at high control transfer while dibutyryl cyclic AMP inhibited water transfer at all levels of control transfer. This was presumably due to the dibutyryl derivative having greater ability to penetrate membranes and to resist degradation by phosphodiesterase (Posternak, Sutherland & Henion, 1962). Our findings are in agreement with other reports that cyclic AMP affects sodium

and water transfer across intestinal epithelia (Field, 1974), abolishing net sodium absorption and replacing it with net sodium secretion (Field, 1971). In this report, at intermediate levels of control transfer, bradykinin and dibutyryl cyclic AMP together produced an inhibition of water transfer which was significantly greater than that seen with dibutyryl cyclic AMP alone. At high control transfer bradykinin and dibutyryl cyclic AMP produced an inhibition of water transfer which was approximately equal to the arithmetic sum of the inhibitory effects of bradykinin and dibutyryl cyclic AMP, possibly due to attainment of a maximum degree of inhibition.

There are reports in the literature that suggest cyclic AMP might mediate the effects of bradykinin in rat thymocytes (Whitfield, MacManus & Gillan, 1970), in fibroblast tissue cultures (Schonhofer, Peters, Karzel, Dinnendahl & Westhofen, 1974) and in guinea-pig lung (Stoner, Manganiello & Vaughan, 1973). However, Schwartz, Kimberg, Sheerin, Field & Said (1974) did not observe any effect of bradykinin on cyclic AMP levels in rabbit ileal mucosa.

Thus in conclusion it has been shown that bradykinin can either stimulate or inhibit water transfer depending on the control level of mucosal water transfer. Theophylline, cyclic AMP and dibutyryl cyclic AMP all mimic the inhibitory action of bradykinin and an interaction was observed between bradykinin and theophylline as well as between bradykinin and dibutyryl cyclic AMP. The results suggest that cyclic AMP may be involved in the inhibitory effect of bradykinin but we have no information concerning the nature of the stimulatory effect of bradykinin.

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