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EFFECT OF CALCIUM AND VASOPRESSIN ON THE RESPONSE OF FROG SKIN TO PROSTAGLANDIN E_1

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SUMMARY

1. Prostaglandin E_1 increases sodium transport as measured by short circuit current (SCC) across isolated frog skin whereas calcium, added to the external Ringer fluid, decreases sodium transport. To help establish the site of action of prostaglandin the possible interaction of these two agents on sodium transport has been examined.

2. The effect of a standard dose of prostaglandin ($0{\cdot}5 \times 10^{-6}$ M) on the short circuit current was tested on paired skins with either zero or high calcium (22·4 mM) in the external Ringer fluid. In ten experiments the responses to prostaglandin (expressed in $\mu A/cm^2$) were not significantly affected by external calcium.

3. In another series of experiments the chelating agent, EGTA, was included in calcium-free external Ringer in order to promote greater depletion of skin calcium. The response of these skins to the standard dose of prostaglandin was of the same order of magnitude as that of control skins. The response was not sustained in contrast to that of normal skins and skins in high-calcium fluids.

4. In a further series of experiments the reverse procedure was adopted whereby the response of the skin to low and high doses of calcium in the external Ringer was recorded in control conditions and when the skin had responded fully to twice the standard dose of prostaglandin. In addition, the calcium-sensitive current was calculated for each skin in both circumstances. The latter was unchanged on addition of prostaglandin, and graded doses of calcium caused the same degree of inhibition of the short circuit current.

5. The results show no interaction between external calcium and prostaglandin and also no need for external calcium in prostaglandin stimulation of sodium transport.

6. The findings do not support the concept of chelation by prostaglandin of calcium from critical sites on the skin as the primary mechanism of its action on sodium transport. The results closely parallel those of a similar type of study into the relationship between vasopressin and external calcium on frog skin also.

7. When frog skin has responded fully to either prostaglandin E_1 or vasopressin, it shows no response to the other, although removal of calcium from the external Ringer fluid causes a further increase in short circuit current.

8. Vasopressin causes a further increase in short circuit current in skins treated with prostaglandin $F_{1\alpha}$. Prostaglandin $F_{1\alpha}$ may be a weaker agonist on frog skin than either vasopressin or prostaglandin E_1.

INTRODUCTION

The primary action of prostaglandin E_1 on frog skin may be a displacement by chelation of calcium ions. The physico-chemical features of the molecule may enable it to do this, as suggested in the guinea-pig uterus (Clegg, Hall & Pickles, 1966) and rat stomach (Coceani, Dreifuss, Puglisi & Wolfe, 1969). The findings of Ramwell & Shaw (1970) that prostaglandin E_1 significantly increases the rate of washout of ^{45}Ca from skins preincubated in ^{45}Ca-containing frog Ringer, strongly suggests possible involvement of this cation in the action of prostaglandin on sodium transport across the tissue. The level of calcium in the solution bathing the epidermal surface of the skin is known to alter the rate of sodium transport as measured by short circuit current across isolated frog skin (Curran & Gill, 1962). The primary effect of calcium appears to be a decrease in the permeability of an outer barrier in the skin to sodium entry (Curran, Herrera & Flanigan, 1963).

Consideration of the above led us to inquire what effect external calcium would have on the response of frog skin to prostaglandin E_1 and also the effect of external calcium on the short circuit current before and after treatment of the skin with prostaglandin. Pertinent to the present studies are the observations of Fassina, Carpenedo & Santi (1969) that a high external calcium decreases the response of the skin of *Rana esculenta* to prostaglandin E_1 and that external calcium is not required for normal prostaglandin action. In the light of the suggestion that prostaglandin E_1 increases sodium transport across frog skin by increasing the permeability of the outer barrier in the skin to sodium entry (Ramwell & Shaw, 1970) and since vasopressin may also increase the permeability of this barrier (Curran *et al.* 1963) it was considered desirable to see if the effects of these two agents on sodium transport are additive.

METHODS

Frogs of the species *Rana temporaria* were used in this study. They were washed daily in tap water and stored, at 6° C, in the dark. The abdominal skin was mounted in an Ussing-type chamber and the short circuit current, the open-circuit potential

and total skin conductance were measured as previously described (Hall, 1973). The skin was bathed on both sides with solutions of the composition shown in Table 1. Other solutions of differing calcium concentrations were prepared by mixing solutions *A* and *B* in the appropriate proportions. In all experiments the skins were bathed on the inside with calcium-free Ringer fluid (solution *A*). It has been shown that frog skin is able to transport sodium normally for long periods when bathed in calcium-free Ringer (Curran & Gill, 1962). The Ringer fluids were continuously mixed by bubbling with moistened oxygen. Prostaglandin E_1 and $F_{1\alpha}$(Upjohn) and vasopressin (Parke Davis) when used were always added to solutions bathing the morphological inside of the skin. Stock solutions of prostaglandin E_1 and $F_{1\alpha}$ were prepared as previously described (Hall, 1973).

TABLE 1. Composition of the calcium-free (solution *A*) and high-calcium Ringer (solution *B*) fluids. Sodium pyruvate (10 mM) was included in the solutions bathing both sides of the skin from late spring and early summer frogs

	Solution *A* (mM)	Solution *B* (mM)
NaCl	69·0	69·0
Choline Cl	44·8	—
$CaCl_2$	—	22·4
KCl	2·0	2·0
$NaHCO_3$	2·4	2·4

RESULTS

This study was carried out on winter, late spring and early summer frogs. Experiments in our laboratory had shown that frogs of the species *Rana temporaria* are partly refractory to prostaglandin (Hall, 1973) during the summer period. Since pyruvate can partly restore the reactivity of such skins to prostaglandin, sodium pyruvate (10 mM) was included in the Ringer fluid for the experiments on late spring and early summer frogs.

Effect of high external calcium on response to prostaglandin E_1

Certain problems arose in the first series of experiments designed to test the effect of external calcium on the ability of frog skin to respond to prostaglandin. The response to prostaglandin is slow, requiring 30 min to reach its peak. After washing out prostaglandin at least 1 h is needed to re-establish the original experimental conditions. Consequently, paired skins were mounted in a twin Ussing-type chamber. In one of the pair the effect of prostaglandin on short circuit current was noted with solution *A* (calcium-free Ringer) on both sides of the skin. In the other skin solution *A* was replaced by solution *B* (high-calcium Ringer) externally. When conditions were stable prostaglandin was added and its effect noted. The dose of prostaglandin selected ($0{\cdot}5 \times 10^{-6}$ M) was previously known to be above threshold but submaximal for most skins in normal conditions.

It is common practice to express the response of frog skin as a percentage of the control short circuit current. Expression of the responses in this way could be somewhat misleading here because high-calcium Ringer externally can frequently halve the resting current and similar responses in paired skins expressed in this manner may consequently appear significantly different. In ten paired experiments the responses to the standard dose of prostaglandin were not significantly altered with high calcium (22·4 mM) externally ($P > 0{\cdot}20$). After 30 min exposure to prostaglandin the mean response in the ten skins with no calcium in the external Ringer fluid was 7·4 μA/cm^2 (Table 2). In the ten skins bathed with high-calcium Ringer externally, the mean response was 8·5 μA/cm^2. Expression of the

TABLE 2. The responses of ten pairs of skins to prostaglandin E_1 ($0{\cdot}5 \times 10^{-6}$ M), with one of the pair in calcium-free Ringer (solution *A*) and the other with high-calcium Ringer (solution *B*) instead of solution *A* on the outside. Application of Student *t* test to the responses (expressed in μA/cm^2) has shown that they do not differ significantly ($P > 0{\cdot}20$). However, statistical treatment of the responses as percentages of the control short circuit currents showed a significant difference ($P < 0{\cdot}01$)

	Calcium-free Ringer on both sides			High-calcium Ringer outside		
Expt no.	Control SCC (μA/cm^2)	Response to PGE_1 (μA/cm^2)	Response as % of control SCC	Control SCC (μA/cm^2)	Response to PGE_1 (μA/cm^2)	Response as % of control SCC
1	17·2	6·4	37	20·4	12	59
2	32·8	6·8	21	21·2	12	57
3	49·2	5·2	11	20·0	4·8	24
4	30·8	9·2	30	13·2	11·6	88
5	19·6	7·2	37	24·8	8·8	35
6	40·8	6·8	17	24·4	6·4	26
7	45·6	10·4	23	10·8	7·2	67
8	31·2	6·4	21	9·6	9·6	100
9	33·6	8·0	24	21·2	4·4	21
10	18·4	7·2	40	15·2	8·0	53
Mean	31·9	7·4	26	18·1	8·5	53

responses as a percentage of the control short circuit current gives a mean response of 53 % in high-calcium conditions and only 26 % with calcium-free Ringer on both sides. Application of the two-sample *t* test has shown these figures to be significantly different ($P < 0{\cdot}01$). This difference arises because the control short circuit current in high-calcium conditions had a mean value of 18·1 μA/cm^2 compared with a mean value of 31·9 μA/cm^2 for the ten skins in calcium-free Ringer. The time course of the response was similar in both experimental conditions.

Effect of zero external calcium on response to prostaglandin E_1

The omission of calcium from the Ringer fluid does not mean that skins bathed in such media lose all their calcium. To promote greater depletion of skin calcium the chelating agent, ethylene glycol bis(β-aminoethylether)-N,N'-tetra acetic acid (EGTA) 1 mM, was included in the calcium-free external Ringer in a series of five experiments. The skins were bathed in this Ringer for 2–3 h before addition of prostaglandin. The responses to prostaglandin were then seen to be of the same order of magnitude as those of skins in high-calcium Ringer fluid. In the experiment shown in Fig. 1

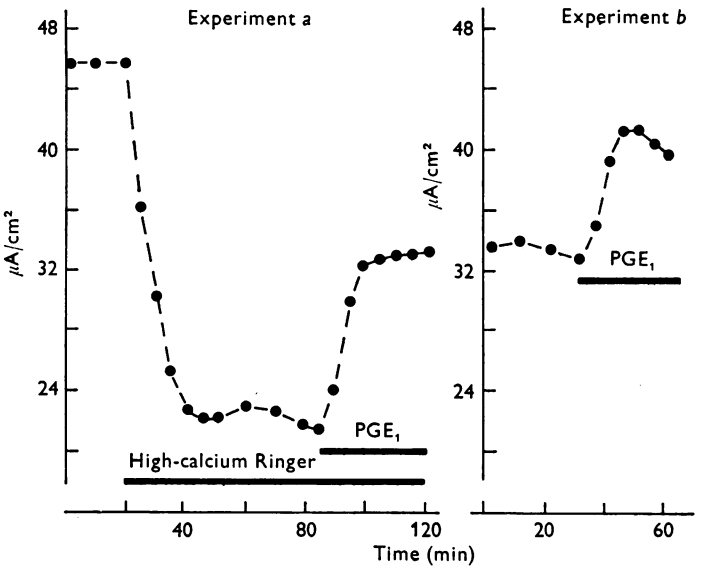

Fig. 1. The response of frog skin to prostaglandin E_1 ($0{\cdot}5 \times 10^{-6}$ M) with high-calcium (22·4 mM) Ringer in experiment *a* and calcium-free Ringer, containing EGTA (1 mM), in experiment *b* bathing the skin externally. Note the transient nature of the response in experiment *b*.

which was representative of the series, the skin bathed in calcium-free Ringer for 3 h with the chelating agent included in the external Ringer, shows a response of the same order of magnitude as a paired skin in high-calcium Ringer. The addition of the chelating agent to the calcium-free Ringer did reveal one difference as shown in Fig. 1. The response of EGTA-treated skins to prostaglandin was not sustained in contrast to that of normal skins and skins in high-calcium Ringer. A similar transient response in frog skins to vasopressin has been reported by Herrera & Curran (1963) and Hong, Park, Park & Kim (1968) in different circumstances. Herrera &

Curran noted that the effect of vasopressin was quite transient in late spring frogs but the effect was sustained if the skins were bathed in a calcium-containing Ringer fluid. There is at present no adequate explanation for this observation but it may indicate a deficiency of calcium at some critical site in the skin.

Effect of prostaglandin E_1 on response to changes in external calcium

In another series of experiments the effect of alterations in external calcium levels on sodium transport in the presence and absence of prostaglandin was observed. Since the effect of alterations in external calcium levels on sodium transport is rapid and easily reversed the effect of calcium could be tested before and after prostaglandin on the same piece of skin. Consequently, in a series of eight experiments each piece of skin served as its own control.

Replacement of solution *A* (calcium-free Ringer) on the outside with high-calcium Ringer fluid causes only a partial inhibition of the short circuit current (Curran & Gill, 1962). The inhibition of this current approaches a limit as calcium is increased above 10 mM and this fractional inhibition of sodium transport (α) is a hyperbolic function of external calcium levels (Curran & Gill, 1962). If the reciprocal of α is plotted against the reciprocal of external calcium concentrations the curve is transformed into a linear form. Extrapolation enables the maximal inhibition achieved at calcium levels approaching infinity to be calculated and this is known as the calcium-sensitive current (Curran & Gill, 1962).

In this series of experiments we selected two concentrations of external calcium, one (11·2 mM) anticipated to give a near maximal effect and the other (1 mM) to give a small but definite response. The experimental design and the results of a representative experiment are shown in Fig. 2. The two concentrations of calcium (1 and 11·2 mM) were tested before the addition of prostaglandin on three electrical variables in the skin, namely short circuit current, skin potential and total skin conductance. When the response to prostaglandin had fully developed, the small and large concentrations of calcium were applied in the same order externally again. It can be seen that the addition of 1 mM calcium to the external solution caused a substantial fall in short circuit current (Fig. 2). The effect is readily reversed by replacement with calcium-free Ringer fluid. The higher calcium dose of 11·2 mM depressed the current to an even greater extent (Fig. 2). The potential across the skin and total skin conductance follow a similar course. The addition of twice the conventional dose of PGE_1 (10^{-6} M) resulted in a considerable stimulation of the short circuit current. The responses now to the two levels of external calcium were of the same order as in the unstimulated skin.

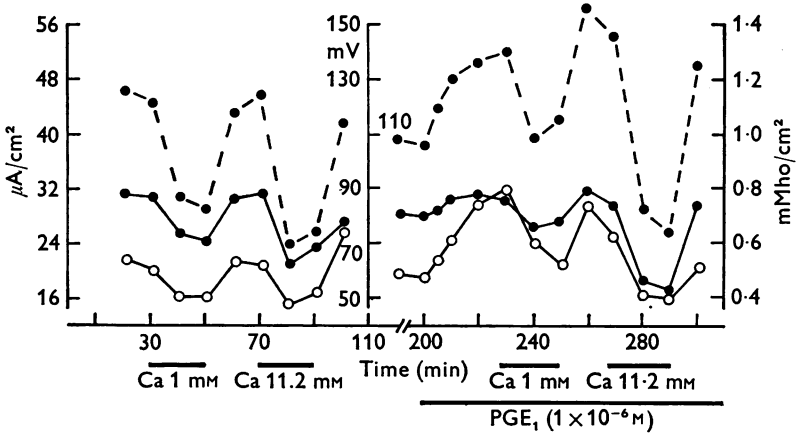

Fig. 2. This Figure shows a representative example of the responses of isolated frog skin to low (1 mM) and high (11·2 mM) calcium Ringer applied externally before and after treatment of the skin with prostaglandin E_1. Three variables were recorded: short circuit current (● — ●), open-circuit potential (●——●) and total skin conductance (○——○).

TABLE 3. The responses of eight skins to low (1 mM) and high (11·2 mM) calcium Ringer fluids applied externally and the calcium-sensitive current, calculated as described in the text after the method of Curran & Gill (1962), in control conditions and when the response to prostaglandin E_1 (10^{-6} M) had fully developed. Application of the one-sample *t* test showed no significant change with prostaglandin

Expt no.	Calcium-sensitive current (μA/cm²)		Calcium 1 mM decrease in SCC (μA/cm²)		Calcium 11·2 mM decrease in SCC (μA/cm²)	
	Control	PG-treated	Control	PG-treated	Control	PG-treated
1	8·0	15·6	2·8	1·6	6·4	8·8
2	22·8	30·4	16·0	12·8	22·0	28·8
3	19·2	12·0	1·6	4·0	10·8	9·6
4	6·8	12·4	4·8	0·8	6·4	6·4
5	14·4	9·6	6·0	4·0	12·8	8·4
6	12·4	12·0	5·6	5·2	8·8	11·2
7	28·8	31·6	7·2	8·0	25·2	27·2
8	10·0	9·6	4·8	4·0	4·8	8·0
	$P > 0{\cdot}50$		$P > 0{\cdot}10$		$P > 0{\cdot}20$	

In each of eight experiments the effects of the two levels of external calcium on short circuit current were measured before application of prostaglandin and when the response to prostaglandin (10^{-6} M) had fully developed (Table 3). The calcium-sensitive current, calculated by plotting the reciprocal of α against the reciprocal of the external calcium levels of 1 and 11·2 mM in both circumstances for each skin, is also shown.

Application of the one-sample t test revealed no significant difference between the paired results. The results are here expressed as absolute values again and not as a percentage or fraction of the control current for the reasons already given. If the effect of calcium is expressed in the conventional manner, an apparently diminished response to calcium may be recorded in prostaglandin-treated skins.

Prostaglandin–vasopressin interaction

Marked seasonal changes in the response of frog skin to prostaglandin E_1 (Hall, 1973) and vasopressin (Hong *et al.* 1968) have been reported with the average response in winter frogs to both agents being similar. Since both agents may act at the same site in the skin the procedure was adopted whereby the skin was exposed to a large dose of vasopressin first and, when the response had fully developed, prostaglandin E_1 was added. The effect of vasopressin on prostaglandin-treated skins was also investigated.

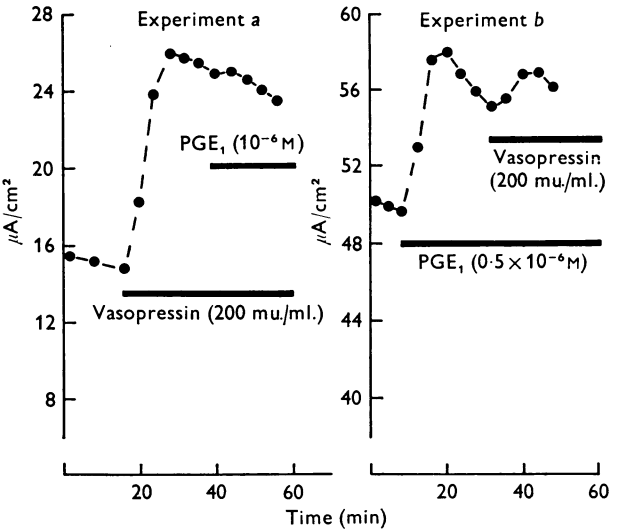

Fig. 3. Prostaglandin E_1–vasopressin interaction on the short circuit current in isolated frog skin. In experiment *a* twice the conventional dose of prostaglandin E_1 had no effect on the short circuit current in a skin pretreated with vasopressin. In experiment *b* the addition of a large dose of vasopressin in the declining phase of the current-response to prostaglandin E_1 caused a response which was somewhat less than the original response to prostaglandin.

The action of prostaglandin E_1 on the short circuit current in a skin treated with a high dose of vasopressin (200 mu./ml.) is shown in Fig. 3. The addition of twice the conventional dose of prostaglandin E_1 had no

significant effect on the short circuit current in this experiment which is representative of a total of six. In the reverse procedure, addition of the standard dose of prostaglandin E_1 increased the short circuit current from 49 to a peak of 58 μA which then gradually declined to a value of about 55 μA (experiment *b*, Fig. 3). The application of vasopressin (200 mu./ml.) at this stage produced a small increase in short circuit current with a peak value (56 μA) less than the prostaglandin peak. In five other experiments the increase in current initiated by vasopressin in prostaglandin-treated skin was either absent or less than the example shown in Fig. 3. It was concluded that if frog skin has responded fully to either prostaglandin or vasopressin, it cannot respond further to either agent.

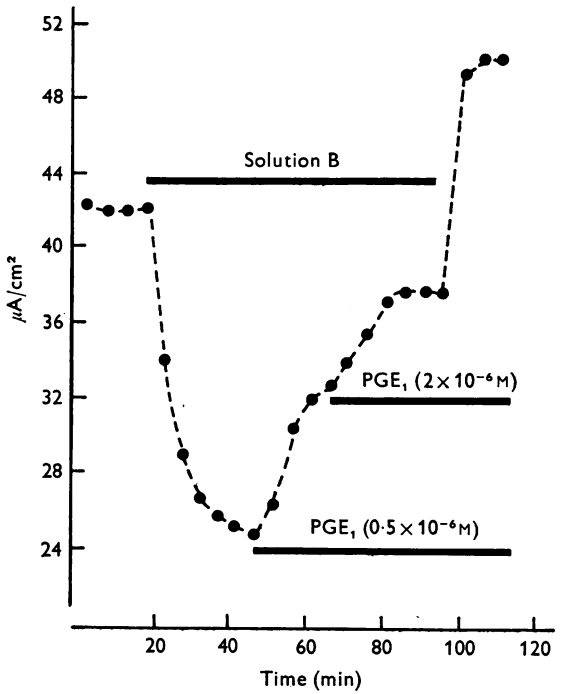

Fig. 4. This type of experiment shows that a skin with high-calcium Ringer (solution *B*) externally and treated with four times the conventional dose of prostaglandin E_1 is still capable of responding to replacement of solution *B* by solution *A* externally.

It could be argued that, at the peak of the response to large doses of either prostaglandin E_1 or vasopressin, the largest increase possible in permeability of the outer barrier to sodium entry has occurred or that the active movement of sodium inwards across the inner barrier is at its maximum. The results from the following type of experiment argue against

this. The replacement of the outside frog Ringer with a high-calcium Ringer results in a fall in short circuit current (Fig. 4). This effect can be rapidly reversed by replacing the high-calcium Ringer in contact with the outside of the skin with a calcium-free Ringer solution. The addition of prostaglandin E_1 to the inside while the skin is bathed with high-calcium Ringer outside results in an increase in short circuit current as in normal circumstances (Fig. 4). As shown in Fig. 4 the addition of the standard dose of prostaglandin E_1 resulted in an increase of about 8 μA in short circuit current and four times the standard dose produced a further increment in current. This finally reached a peak of 38 μA from a starting value of 25 μA, an increase of about 52 %. The replacement of the high-calcium Ringer bathing the outside of the skin with calcium-free Ringer at the peak of the response to four times the conventional dose of prostaglandin E_1 resulted in a further increase of about 12 μA in short circuit current.

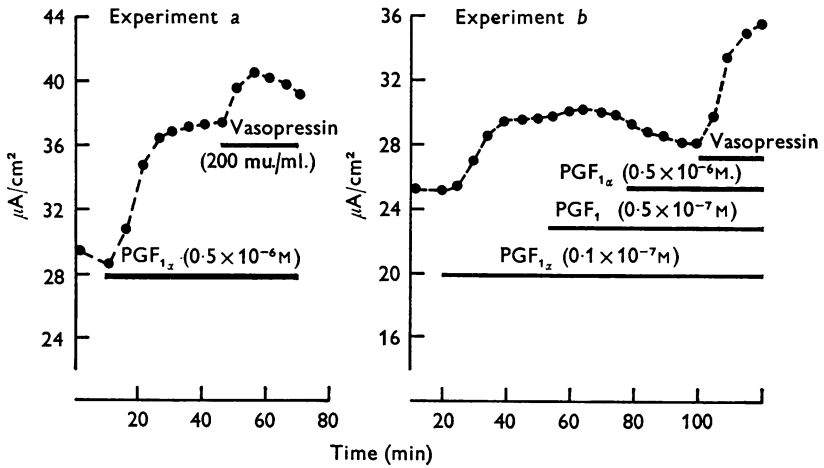

Fig. 5. Prostaglandin $F_{1\alpha}$–vasopressin interaction on the short circuit current in isolated frog skin. In experiment *a* vasopressin produced a substantial increase in short circuit current at the peak of the response to prostaglandin $F_{1\alpha}$. In experiment *b* progressively larger doses of prostaglandin $F_{1\alpha}$ were added until the maximum effect by it was achieved. The addition of vasopressin (200 mu./ml.) at this stage produced a further increase on the existing response to prostaglandin $F_{1\alpha}$.

In winter frogs the average increase in current in response to prostaglandin $F_{1\alpha}$ was less than the mean response to prostaglandin E_1 (Hall, 1973). In skins treated with large doses of vasopressin (200 mu./ml.), prostaglandin $F_{1\alpha}$ had no additional effect on the short circuit current. In skins exposed to the standard dose of prostaglandin $F_{1\alpha}$ ($0{\cdot}5 \times 10^{-6}$ M), vasopressin (200 mu./ml.) produced a significant increase in short circuit current. This

is shown in a fairly typical experiment in Fig. 5, where vasopressin produced a further increment of 3 μA on an existing response of 8 μA to the standard dose of prostaglandin $F_{1\alpha}$. It is conceivable that the dose of prostaglandin $F_{1\alpha}$ in experiment *a*, Fig. 5, was submaximal and the subsequent response to vasopressin could be explained as a simple additive effect. However, in experiment *b*, Fig. 5, the addition of progressively larger doses of prostaglandin $F_{1\alpha}$ without wash-out until no further increment in short circuit current occurred, ensured that the maximum dose of prostaglandin $F_{1\alpha}$ had been exceeded. In this skin the addition of vasopressin at this stage caused a further increase of 7 μA on the existing response to supramaximal levels of prostaglandin $F_{1\alpha}$.

DISCUSSION

The calcium-prostaglandin studies showed no competition between these agents on sodium transport and no requirement for calcium in the external medium. That both agents act independently can only be explained by a parallel arrangement of their sites of action. If the sites were arranged in series, the prostaglandin-activated part of sodium transport would be sensitive to treatment of the skin with calcium since calcium acts on the outer barrier (Curran *et al.* 1963). Consequently, they would appear to interact even though direct competition did not exist between them. It is further possible that the sites of action for these two agents may be located on the same barrier to sodium transport. In a similar type of study Herrera & Curran (1963) noted that calcium and vasopressin do not compete but act independently on sodium transport in frog skin at two different sites. These sites appear to be located on the outer barrier to sodium entry in the skin (Curran *et al.* 1963). The findings of Ramwell & Shaw (1970) are in agreement with the view that prostaglandin acts on this outer barrier.

That there is no need of external calcium for normal prostaglandin action on the short circuit current agrees with the findings of Fassina *et al.* (1969). Herrera & Curran (1963) also observed substantial increases in the current in frog skins with no external calcium when treated with vasopressin. Experiments on toad bladder have indicated a requirement for calcium in order to obtain an increase in current on addition of vasopressin (Bentley, 1960). This apparent discrepancy between toad bladder and frog skin may reflect an ability of frog skin to retain sufficient calcium for normal responses when bathed with calcium-free Ringer fluids. In our experiments the addition of the chelating agent, EGTA, to the calcium-free Ringer bathing the epidermal surface of the skin for long periods, did not block the action of prostaglandin on sodium transport. It did change the normal character of the response to a transient form as noted in

summer frogs with vasopressin (Hong *et al.* 1968). The non-dependence of the magnitude of the response to prostaglandin on external calcium mimics the findings with vasopressin and does not lend support to the idea that prostaglandin acts primarily by chelating calcium at certain critical sites in the outer barrier of the skin.

The transient nature of the skin response to prostaglandin after treatment with a chelating agent in the external Ringer fluid may indicate a deficiency of calcium at some further stage in the sodium transport system. It is known, for example, that normal sodium transport is decreased if a chelating agent is added to the Ringer fluid bathing the morphological inside of the skin (Curran, Zadunaisky & Gill, 1961). Apparently some calcium is needed on the inside if the skin is to retain its normal properties.

Our findings that the short circuit current response to prostaglandin is unaffected by high levels of external calcium differ from those of Fassina *et al.* (1969). They report a decrease in the response of the skin of *Rana esculenta* to prostaglandin in the presence of high external calcium. To explain this difference between our results and those of Fassina and his co-workers is not easy. A possible explanation of the difference is a species variation. In this regard it may be of significance that in *Rana temporaria* there was a marked response to prostaglandin down to an external sodium level of 7 mM (Hall, 1973) whereas the effect of prostaglandin was abolished in the skin of *Rana esculenta* when the normal Ringer fluid was replaced externally by 1/10 frog Ringer. It has recently been suggested (Cereijido & Rotunno, 1968) that in addition to transcellular sodium transport, an intercellular mode of transport exists in frog skin. Polar groups in the outer leaflets of lipid in the membranes of surface cells form a system of fixed charges to bind sodium ions which are then shunted from site to site to reach pumping sites facing intercellular spaces. The proportion of sodium transported by either route may vary from one species of frog to the next and may also depend on the experimental circumstances. Calcium may act on sodium transport by reducing the permeability of the outer cell membranes to sodium and in this way reduce the sodium available to the pumping sites. Prostaglandin may act by increasing the permeability of these membranes and in circumstances where the external sodium is high, more sodium becomes available at the pumping sites. In such circumstances high calcium would diminish the response to prostaglandin as apparently happens in *Rana esculenta*.

In other skins prostaglandin may largely act by stimulating intercellular transport. This means of transport and stimulation thereof should still be apparent at quite low external sodium levels and this appears to be the case with *Rana temporaria* (Hall, 1973). It is also necessary to postulate that this means of transport is independent of external calcium.

As with prostaglandin in the present study Herrera & Curran (1963) found no interaction between calcium and vasopressin on the skin of *Rana pipiens*.

Further evidence for the independence of calcium and prostaglandin actions on the skin of *Rana temporaria* has been provided by the study of Hall & O'Regan (1973). Frog skin can be excited by rectangular-wave electrical stimuli (Finkelstein, 1964). The spike-shaped transient which occurs is considerably elongated by external calcium while it is unaffected by prostaglandin. The spike appears to be generated across the outer membrane of the surface cells (Lindemann & Thorns, 1967).

The short circuit current-reponse to prostaglandin E_1 in toad bladder amounts to only 40% of the response to vasopressin (Lipson & Sharp, 1971) while in frog skin the average response to E_1 in winter frogs (Hall, 1973) was similar to that reported by Hong *et al.* (1968) for vasopressin. The present study shows that when frog skin has responded fully to either prostaglandin or vasopressin it cannot react to the other. At the peak of the response to either compound, a further increase in permeability of the outer barrier to sodium entry can occur if, for example, external calcium is removed with an increase in the rate of sodium transfer across the inner barrier also. Thus the lack of response to vasopressin at the peak of a maximal response to prostaglandin E_1 or vice versa is not due to the maximum change in permeability of the outer barrier or the maximum rate of sodium transfer across the inner barrier having been achieved. The findings support the suggestion that prostaglandin E_1 and vasopressin act at the same site because the maximal effect of either cannot be augmented by the other. In the case of prostaglandin $F_{1\alpha}$, vasopressin causes a further increment in the current-response indicating that the peptide is a more powerful agonist, if they both act at the same site. It is of interest that the mean response in winter frogs to prostaglandin $F_{1\alpha}$ was 31% (Hall, 1973) compared with 47% in the case of vasopressin (Hong *et al.* 1968).

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