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Action of Caerulein, Gastrin 17, Pentagastrin, and Secretin on the Active Transport of Sodium by the Frog Skin

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Summary. Frog skin was mounted in an Ussing chamber and the actions of caerulein, gastrin, pentagastrin, and secretin on the active transport of sodium were studied using the short-circuit current method. All polypeptides exerted their effect when placed in the solution bathing the outside surface of the skin. The response was a transient dose-related increase in the transepithelial electrical potential difference and in the short-circuit current. Analysis of the response indicated that at submaximal doses the effect was due to an increase in the rate of entry of sodium through the outer barrier to active sodium transport. At supramaximal doses the passive permeability of the skin was also increased. Th ED50 concentrations of the hormones were: caerulein, 50 pM; gastrin, 53 pM; pentagastrin, 440 pM; and secretin, 30 pM. It is argued that the large quantity of caerulein or caerulein-like peptides stored in the skin may be required either to control the entry of sodium when the amphibian is undergoing maximum stress in a freshwater environment, or that it may have a protective function for the amphibian as it could elicit a noxious hypersecretion in the gastrointestinal tract of the predator together with a marked hypotension.

Key words: Frog skin, caerulein, gastrin, pentagastrin, secretin, transport.

Many small polypeptides have been isolated from the skins of amphibia. These peptides have displayed a wide range of pharmacological actions on vascular and extravascular smooth muscles and also potent stimulating action on gastrointestinal tract secretions. In particular, caerulein, a decapeptide isolated by Anastasi, Erspamer and Endean (1968) from the Australian amphibian *Hyla caerulea,* has a C-terminal ending which is remarkably similar to the C-terminal ending of gastrin and cholecystokinin-pancreozymin. Its effects in the gastrointestinal tract are similar to the actions of these two hormones. It causes contraction of the guinea-pig gallbladder with a threshold dose of 0.2–0.6 ng/kg body weight and stimulates gastric acid secretion in the conscious dog above a threshold dose of 0.1–0.5 μg/kg body weight (Erspamer et al., 1967). The potency of caerulein and its ready availability in synthetic form have led to its introduction as a stimulant in routine clinical testing of human gastrointestinal function. However, in spite of the availability of caerulein, its potent effects on active transport in the gastrointestinal tract, and its natural occurrence in amphibian skin, the effect of this decapeptide on the active transport mechanisms in amphibian skin has not been determined. Therefore we decided to investigate this effect, and, in view of the marked similarity of the C-terminal endings, to also test a range of gastrointestinal hormones and fragments on the frog skin.

Materials and Methods

The experiments were carried out in the months of June and July on *Rana pipiens* (Xenopus Ltd., South Nutfield, Redhill, Surrey, England). In this species of frog the short-circuit current can be equated to the active transport of sodium ions, as the active transport of chloride ions is negligible. The frogs were pithed, and segments of either ventral or dorsal abdominal skin were mounted in Ussing chambers (Ussing & Zerahn, 1951). A circular area of 1.65 cm^2 was exposed to the bicarbonate buffered Ringer's bathing solution (NaCl, 90 mM; $NaHCO_3$, 20 mM; KCl, 2 mM; $MgCl_2$, 1.0 mM; $CaCl_2$, 1.1 mM; glucose 2 g/liter) which had been equilibrated with 95% oxygen/5% carbon dioxide before introduction into the chambers.

The hormones caerulein (Sigma Chemical Company Ltd., Poole, Dorset, England); natural gastrin 17 (gift from Professor R.A. Gregory, Physiological Laboratory, University of Liverpool, England); pentagastrin (ICI 50123) (ICI Ltd., Alderley Park, Macclesfield, Cheshire, England), and synthetic porcine secretin (Roche Products Ltd., Welwyn Garden City, Herts, England) were diluted in the gassed Ringer's solution immediately before application to the skins.

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Electrical Measurements

The transepithelial electrical potential difference was measured using Ag/AgCl reversible electrodes which were electrolytically coated on the day of the experiment and mounted in polyethylene tubes containing 2% wt/vol agar made up in the bicarbonate Ringer's solution. Electrode assemblies with difference potentials of greater than 1 mV when placed in the same solution were discarded. These potential measuring electrodes were placed in close proximity to the skin surfaces. The current-carrying electrodes were large coils of Ag/AgCl-coated wire at the distal ends of the mounting chambers. Measurements of short-circuit current and transepithelial electrical potential difference were made using an automatic device which maintained the short-circuit condition for 48 sec then switched to measurement of transepithelial electrical potential difference for the remaining 12 sec of a 1-min repetitive cycle. The skin was maintained in the short-circuit condition for the majority of the time because the active sodium transport rate is voltage sensitive (Biber & Sanders, 1973). Standardizing the electrical potential at zero will thus reduce the variation between skins. The output from the automatic device was recorded on a Bryans Southern Instruments 28000 single-channel potentiometric recorder. The baselines of both parameters were superimposed, and the recordings of potential and short-circuit current could easily be distinguished by their respective characteristic dot and dash appearance.

The dose-response relationships of the polypeptides were analyzed using a FORTRAN development of the BASIC routines for logistic curve fitting by De Lean, Munson and Rodbard (1978). Results in the text are presented as mean ± SE (number of observations).

Results

In the initial experiments the polypeptides were applied separately in the inside or outside bathing solutions. For all of the peptides tested the effect was immediate in onset and reproducible only when the peptides were added to the solution bathing the outside of the skin. Responses to peptides applied in the solution bathing the inside surface of the skin occurred only when the peptide concentration was much higher than that required to stimulate on the outside surface, took considerably longer before a change in the electrical parameters of the skin occurred, and such changes as did occur were variable in magnitude and direction. From these results it was decided that the receptor sites with which the polypeptides interacted were situated on the outward facing barrier of the epithelium, and in all later experiments the polypeptides were only applied to this surface.

The electrical response of the frog skin to all of the polypeptides tested was a time coincident transient increase in transepithelial electrical potential difference and short-circuit current across the skin (Fig. 1). The magnitude and duration of the responses were dose dependent. The time course of the response to a half-maximal dose was a rise to a peak at $7.1 \pm 0.2(69)$ min, followed by a decline to the prestimulation level in $35.5 \pm 1.0(69)$ min. It was assumed that this decline was due to the breakdown of the polypeptide into an inactive form as a subsequent application of the polypeptide into the same solution elicited a dose-dependent electrical response. In the investigation of the dose dependency of the response, the bathing solution was always replaced with freshly oxygenated Ringer's bicarbonate solution between the stimulations, and the dosage was altered in a randomized manner.

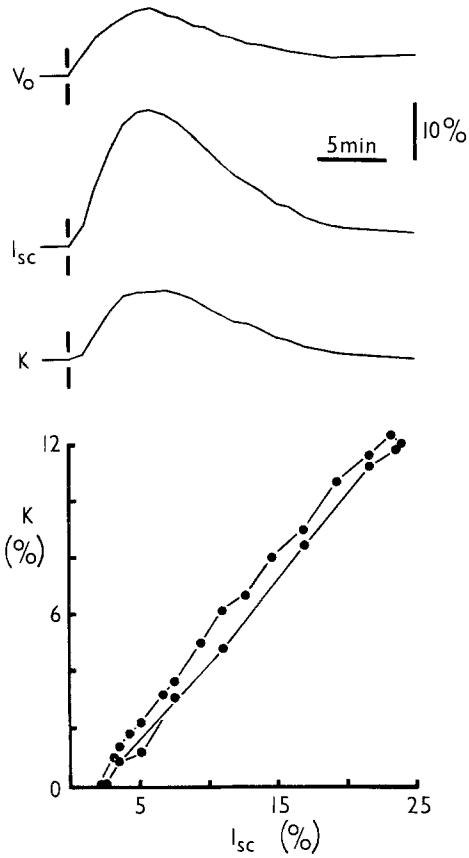

Fig. 1. The action of 37 pM Caerulein applied in the solution bathing the outer surface on the electrical properties of the frog skin. In the upper section of the figure the time course of the % change from the prestimulation value of the open circuit electrical potential difference (V_o), the short-circuit current (I_{sc}), and the total transepithelial electrical conductance (K) are shown. The initial values of the parameters were $V_o = 59$ mV; $I_{sc} = 25.8\ \mu A \cdot cm^{-2}$; $K = 0.437$ mMho $\cdot cm^{-2}$. In the lower section of the figure the change in conductance is plotted against the change in short-circuit current. Analysis of this graph using the method of Yonath and Civan (1971) indicates that the conductance of the active transport pathway is altered by the application of caerulein

Occasionally a second peak was observed about one hour after the addition of the polypeptide to the bathing solution, but the characteristics of this peak were not investigated. A similar second peak has been observed by Tomlinson and Wood (1976) in the response of the frog skin to catecholamines.

The results were analyzed further using the simple electrical equivalent circuit model introduced by Ussing and Windhager (1964) and elaborated by Yonath

Table 1. The dose-response data for caerulein, gastrin, pentagastrin, and secretin[a]

Dose (pM)	V_o (mV)	$\%V_o$ (%)	I_{sc} ($\mu A \cdot cm^{-2}$)	$\%I_{sc}$ (%)	Dose (pM)	V_o (mV)	$\%V_o$ (%)	I_{sc} ($\mu A \cdot cm^{-2}$)	$\%I_{sc}$ (%)
Caerulein					Pentagastrin				
1.0	46.2	1.3	26.8	2.0	13.0	56.5	1.4	40.2	2.5
2.0	62.2	1.5	30.3	4.5	26.0	44.4	1.5	19.1	2.1
7.0	53.0	3.9	29.4	6.4	26.0	54.4	4.2	23.9	6.1
7.0	58.4	5.2	22.1	8.5	65.0	49.1	2.2	41.2	3.6
9.0	61.5	3.6	29.6	10.4	65.0	37.0	3.6	26.6	5.5
9.0	73.3	3.4	32.9	5.1	65.0	57.3	6.1	36.1	9.4
9.0	60.5	3.7	27.8	6.3	130.0	80.5	3.2	34.7	6.6
9.0	51.0	5.7	26.0	9.2	130.0	65.0	6.8	22.5	10.5
9.0	60.0	4.4	26.7	9.3	260.0	43.9	6.2	18.1	10.0
18.0	58.8	4.2	21.2	12.3	260.0	43.5	8.2	37.4	14.9
46.0	55.5	8.5	26.6	12.3	330.0	45.5	4.7	29.1	16.3
46.0	49.0	10.5	23.6	16.1	650.0	51.2	5.4	23.8	16.5
74.0	69.0	7.1	27.3	19.6	1300.0	46.8	5.4	28.5	26.5
93.0	58.0	7.7	25.4	19.7	1300.0	71.6	11.4	24.9	18.6
93.0	70.0	7.1	27.0	14.0					
93.0	62.0	9.7	30.6	23.3	Secretin				
93.0	51.2	6.6	25.4	19.5	0.3	65.0	1.8	20.5	3.5
93.0	69.0	6.9	30.9	17.8	1.6	79.6	4.4	38.0	9.4
740.0	65.0	10.6	26.7	27.3	1.6	83.4	2.6	31.1	8.1
					2.5	56.3	3.3	51.4	8.4
Gastrin					3.3	51.5	2.5	21.1	5.4
5.0	65.8	1.3	21.4	2.2	7.0	82.5	1.2	32.5	10.3
9.0	57.3	1.9	45.9	3.5	7.0	45.4	2.5	21.6	11.2
9.0	67.0	3.0	25.7	7.0	10.0	74.7	2.7	28.1	10.2
23.0	78.3	4.5	30.7	10.0	10.0	67.1	5.1	29.1	9.5
23.0	48.7	5.8	39.9	11.2	16.0	64.0	3.2	38.3	15.6
46.0	65.0	9.4	27.9	14.5	16.0	77.5	8.7	31.9	16.6
46.0	50.0	8.6	20.1	13.3	25.0	68.7	2.8	28.6	10.4
46.0	56.0	5.3	24.3	17.3	33.0	82.0	4.8	33.4	18.3
46.0	62.8	6.8	22.9	16.6	33.0	70.7	3.8	25.1	17.1
93.0	84.9	7.2	20.3	19.4	33.0	68.4	4.4	35.3	20.4
93.0	56.0	6.7	29.3	15.2	65.0	73.7	8.7	28.8	17.2
93.0	62.7	11.4	33.9	23.5	82.0	92.9	3.6	32.9	18.2
230.0	77.6	11.6	35.7	23.5	100.0	62.0	11.3	32.2	20.9
230.0	59.8	11.8	38.4	26.4	330.0	48.6	12.5	33.2	22.7
460.0	85.9	8.6	37.8	28.0	330.0	52.2	14.7	41.9	23.0
					3300.0	65.0	9.3	29.6	28.2

[a] V_o is the prestimulation transepithelial electrical potential difference and I_{sc} the prestimulation short-circuit current. The maximum change in these parameters on application of the polypeptides is expressed as a % of these prestimulation values, $\%V_o$ and $\%I_{sc}$, respectively.

and Civan (1971). This treatment is approximate, as it assumes the properties are linear and not voltage sensitive. In this model the active transport system is represented by the series arrangement of an electromotive force driving the active sodium transport and a conductance limiting the current flow through this pathway. The leakage shunt pathway through the skin is represented by a conductance in parallel with the active transport elements.

This analysis indicated that for submaximal doses the observed changes in the electrical parameters were consistent with an alteration in the conductance element in the active transport pathway. At supramaximal doses the conductance-current relationship departed from linearity, indicating a possible increase in the leakage conductance at these higher doses. Throughout the dose range investigated there was no indication of an alteration in the electromotive force.

In any response the maximum change in the short-circuit current was always greater than the maximum change in the transepithelial electrical potential difference. The short-circuit current dose-response data also showed a smaller experimental variation about the mean sigmoid curve. For these reasons the analysis was confined to an investigation of the dose-response relationship of the maximum change in short-circuit current occurring in response to application

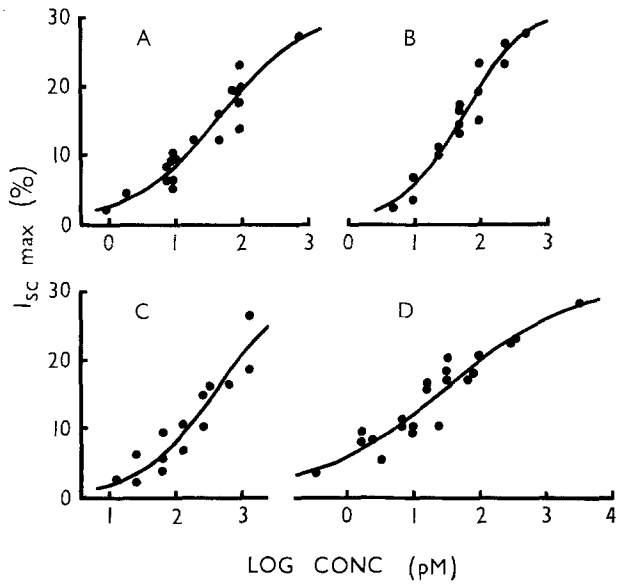

Fig. 2. The log dose-response relationships for the polypeptides investigated. The maximum % change in the short-circuit current is plotted against the logarithm of the molal concentration of (*A*) caerulein, (*B*) gastrin 17, (*C*) pentagastrin, and (*D*) secretin in the outside bathing solution. The least squares best fit logistic function was obtained by a modification of the method of De Lean et al. (1978). The ED50 concentrations are 50, 53, 440, and 30 pM, respectively

of the polypeptides. A further improvement in the analysis was effected by normalizing the response, expressing the peak change in short-circuit current as a percentage of the prestimulation value. The results were analyzed using a development of the De Lean et al. (1978) sigmoid curve-fitting program. With all minimal responses equated to zero, it was observed that the maximal responses for all the polypeptides were not statistically significantly different ($P > 0.05$), so the maximal responses were constrained to be equal. With these constraints the maximal change in the short-circuit current was 31.8 ± 2.8 (69) %, the relative potencies were caerulein/gastrin/pentagastrin/secretin = 1.00(19): 0.94 ± 0.18(15): 0.11 ± 0.02(14): 1.64 ± 0.43(21) with ED50's of 50:53:440:30 pM, respectively (Fig. 2, Table 1).

Discussion

The mechanical clamping of the frog skin in the apparatus produces a limited amount of tissue damage localized to a small zone at the clamped edge. This effect was investigated by Dobson and Kidder (1968) who found that near the clamped edge the intercellular spaces were enlarged and the passive leakage conductance increased. This damage gave a reduction in the measured transepithelial electrical potential difference, but did not alter the short-circuit current. The magnitude of the potential difference reduction was dependent on the edge length to surface area ratio. In our apparatus this ratio was 2.76 cm^{-1} which would give an approximately 10% reduction in the measured potential.

Koefoed-Johnsen and Ussing (1958) proposed a double-barrier model for the frog skin. Subsequent experiments, which used glass microelectrodes to record the electrical potential profile through the skin, have to a large extent confirmed this double-barrier model since there were usually two distinct steps in the recording as the microelectrode was traversed through the frog skin (Cereijido & Curran, 1965; Biber & Curran, 1970; Nagel, 1978). The outer barrier accounts for 75% of the ohmic resistance in the active transport pathway (Helman & Fisher, 1977), though this proportion may be the effect of the lower surface area of the outward facing barrier, compared to the deeply invaginated larger area of the inward facing barrier, rather than to a marked difference in the resistance per unit cell membrane area.

The transport of sodium ions across the outward facing barrier is the rate limiting step in the active transport process, with the limitations being due either to a saturation of a carrier mechanism (Biber & Curran, 1970; Biber, 1971; Biber & Sanders, 1973; Erlij & Smith, 1973; Cruz & Biber, 1976), or a limit to the number of pores available for the electrodiffusion of sodium (Fuchs, Larsen & Lindemann, 1977; Lindemann & Van Driessche, 1977). The outer barrier is near the anatomical outside (Kidder, Cereijido & Curran, 1964). Inside this barrier the living cells function as a syncytium with low resistance junctions between the cells, permitting a relatively free flow of sodium ions from cell to cell. The second barrier to sodium transport is located at the junction of these living cells and the large interdigitating intercellular spaces which communicate freely with the inside bathing solution. This inner barrier is the site of an active sodium transporting mechanism which has been demonstrated electrically (Helman & Fisher, 1977) and by the use of tritiated ouabain (Mills, Ernst & DiBona, 1977).

As the polypeptides produced an immediate response at lowest concentration when applied in the outside solution and as this outer barrier is superficial, it is suggested that the action of the polypeptides investigated is solely on this outward facing barrier. The action of the polypeptides is to increase either the number of active carrier sites postulated by Biber and co-workers or to open more of the pores postulated by Lindemann and co-workers. In this way the passage of sodium ions through this rate limiting barrier is modulated. The polypeptides apparently do not act directly on the active sodium transporting

mechanism sited on the inward facing barrier, since they are not effective when applied to this surface of the skin.

It is probable that an alteration in the rate of transport of other ions contributes to the observed increase in the short-circuit current stimulated by the polypeptides, though this contribution would be small since sodium is the major actively transported ion.

The site of action must either be relatively nonspecific or there may be two or more receptors, for the C-terminal ending of secretin is considerably different from the C-terminal endings of the other polypeptides. It is interesting to note that the relative potencies of gastrin and pentagastrin in their action on frog skin (0.94:0.11) are remarkably similar to their relative potencies in the stimulation of gastric acid secretion in conscious gastric fistula cats (1.0:0.09) (Hirst, Reed & Shaw, 1977).

The mode of action is different from that of antidiuretic hormone (ADH), which acts only from the inside bathing solution and elicits a gradual increase in transepithelial electrical potential difference and short-circuit current which both rise to a plateau in 30 min after ADH application. The action of ADH is thought to be mediated via the second messenger cyclic-AMP which traverses the cell to give an increase in the permeability to sodium and water at the outward facing barrier.

In the amphibian skin, caerulein and caerulein-like polypeptides are stored in granules in dermal glands. These glands contract to expel their contents into the outside solution when either the nerve supply to the skin is electrically stimulated or noradrenaline is applied (Dockray & Hopkins, 1975). The spread of the caerulein-like polypeptides would produce an alteration in the electrical parameters of the skin secondary to any direct effect of the catecholamine. The electrical responses of toad skin to noradrenaline (House, 1970) bear such a marked resemblance to the responses to direct stimulation of frog skin with caerulein that it is tempting to suggest what has until now been regarded as the response to catecholamine stimulation is in fact the response to polypeptides released from the skin in response to catecholamine stimulation.

The quantity of caerulein required to elicit an alteration in the active transport of sodium across the frog skin was of the order of nanograms per square centimeter. Anastasi et al. (1968) note that the average *Hyla caerulea* skin contains a total of 1.2–2.0 mg of caerulein. This large quantity of stored peptide may be required to control sodium reabsorption when the frog is undergoing maximum sodium loss stress in a freshwater environment. The mucus layer on the skin presents a negligible barrier to the diffusion of the polypeptides examined in this paper, so a large release of the polypeptide would be required to maintain the concentration at the skin surface in the suprathreshold range. Amphibia may have evolved this adrenergically influenced hormonal control of the sodium permeability at the outer barrier as an alternative to the mode of action of ADH on the inner cell membrane, when a secondary messenger must cross the cell to alter the permeability of the outer barrier. An alternative hypothesis to explain the storage of such a large quantity of polypeptide is to assign a protective function to the hormone. Provided the decapeptide was absorbed from the gastrointestinal tract of a predator species, the ingestion of these amphibia could produce a massive and noxious stimulation of the gastrointestinal tract and hypotension in the predator, for as Anastasi et al. (1968) observed, the skin of only one *Hyla caerulea* contained sufficient caerulein to study the gallbladder function of 5,000–10,000 humans.

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