Biochimica et Biophysica Acta, 1136 (1992) 175–180
© 1992 Elsevier Science Publishers B.V. All rights reserved 0167-4889/92/$05.00

BBAMCR 13204

Endothelin increases $[Ca^{2+}]_i$ in rat pancreatic acinar cells by intracellular release but fails to increase amylase secretion

David I. Yule, George T. Blevins Jr., Andreas C. Wagner and John A. Williams

Department of Physiology, University of Michigan, Ann Arbor, MI (USA)

(Received 23 March 1992)

Key words: Pancreatic acinar cell; Endothelin; Intracellular calcium ion

In individual fura-2 loaded cells of rat pancreatic acini endothelin-1 (ET-1) (10–50 nM) induced sustained oscillations in $[Ca^{2+}]_i$. At higher concentrations a larger, but transient increase in $[Ca^{2+}]_i$ was observed, which was largely unaffected by removal of extracellular Ca^{2+}. ET-1 induced the release of Ca_i^{2+} from the same store as cholecystokinin (CCK), but with less potency. At concentrations of endothelin which transiently increased Ca^{2+}, ET-1 increased the accumulation of inositol phosphates. Specific binding sites for ^{125}I-endothelin were demonstrated on rat pancreatic acini. A single class of binding sites was identified with an apparent K_d 108 ± 12 pM and B_{max} of 171 ± 17 fmol/mg for ET-1. The relative potency order for displacing [^{125}I]ET was ET-1 > ET-2 > ET-3. In contrast to CCK and the non-phorbol ester tumour promoter Thapsigargin (TG) which induce both transient and sustained components of $[Ca^{2+}]_i$ elevation, ET-1 failed to increase amylase release over the range 100 pM–1 μM.

Introduction

Recently, much interest has been shown in the 21 amino-acid peptide termed endothelin (ET). This peptide, originally isolated from the culture medium of porcine aortic endothelial cells, is synthesized and secreted by vascular endothelial cells and has been shown to be the most potent endogenous vasopressor substance yet discovered [1,2]. Three genes have been found in human DNA, which code for different isoforms of endothelin, denoted ET-1, ET-2 and ET-3 [3]. The endothelins are synthesised as isoform specific pre-pro-peptides of approx. 200 amino acids, which are proteolytically cleaved to form a 38–39 residue 'Big' endothelin. A proteinase then cleaves 'Big' ET to give the mature endothelin.

Plasma levels of ET are reported to be below that which would cause vasoconstriction and, thus, it is thought unlikely to function as a circulating vasoregulatory hormone. It has been proposed that ET could function as a paracrine signal released locally to influence the underlying smooth muscle tone [4]. Since receptors have been localised not only on the vasculature but on a diverse variety of tissues including the brain, adrenal glands, lung, kidney and liver [5,6], it is possible that ET could have a wider role, functioning as a local regulator in these tissues. Indeed, ET has been shown in 3T3 fibroblasts and cultured mesengial cells to be a potent mitogen, stimulating c-*fos* and c-*myc* proto-oncogene expression [7,8]. ET receptors are also present in the CNS and ET can cause noradrenaline release from nerve endings [9].

Recently, ET has been shown to act on cells isolated from pancreatic arterioles to increase $[Ca^{2+}]_i$ (with permission, personal communication D. Storm, E. Stuenkel and C. Webb). As ET acting on arteriolar muscle cells might also interact with the parenchymal cells of the pancreas, we have investigated the possibility of ET action directly on pancreatic acinar cells.

Materials and Methods

Materials

Endothelins were obtained from Bachem (Torrance, CA), fura-2 AM from Molecular Probes (Eugene, OR) and thapsigargin from L.C. Services (Boston, MA), [^{3}H]inositol and 3-[^{125}I]-iodotyrosyl endothelin-1 from Amersham (Arlington Heights, IL). Unless otherwise stated, all other materials were from Sigma (St. Louis, MO).

Methods

The methods used are essentially similar to those previously described [10–12]. In brief, pancreata were excised from fed adult male Sprague-Dawley rats (200–250g) and acini prepared by enzymatic digestion with

Correspondence to: D.I. Yule, Department of Physiology, University of Michigan, 7744 Medical Science II Ann Arbor, MI, USA.

collagenase [10]. Acini were then suspended in a physiological salt solution (PSS) containing 10 mg/ml BSA, 0.1 mg/ml soybean trypsin inhibitor (SBTI) and (in mM): NaCl (137), KCl (4.7), $MgCl_2$ (0.56), $CaCl_2$ (1.28), Na_2HPO_4 (1.0), Hepes (10), L-glutamine (2) and D-glucose (5.5). The pH was adjusted to 7.4 and equilibrated with 100% O_2. For fluorescence measurements the PSS was identical, except that BSA and SBTI were omitted. To prepare Ca^{2+}-free media, $CaCl_2$ was omitted from the PSS and 1 mM EGTA was added.

Measurement of $[Ca^{2+}]_i$

Isolated acini were incubated with 1 μM fura-2 AM at ambient temperature for 30 min and then washed and resuspended in fresh PSS. For measurement of intracellular Ca^{2+}, fura-2 loaded acini were transferred to a closed chamber, mounted on the stage of a Nikon Diaphot microscope, and continuously superfused at 1 ml/min with 37°C PSS [12]. Solution changes were rapidly accomplished by means of a valve attached to an 8-chamber superfusion reservoir. Individual cells protruding from an acinus were isolated optically by means of a pin-hole diaphragm. Dual excitation, alternating between 340 and 380 nm, was provided by a SPEX fluorolog system (SPEX, Edison, NJ.) and emission at 505 nm associated with this excitation was stored and analysed by DM3000cm software (SPEX). Calibration of fluorescent ratio signals was accomplished as previously described according to the equation of Grienkiewicz et al. [13], in which $[Ca^{2+}] = k_d \cdot \beta \cdot \{R - R_{min}\}/\{R_{max} - R\} \cdot R_{max}$, R_{min} and β are 11.4, 0.64 and 8.125, respectively.

Measurement of [^{3}H]inositol phosphates

For the measurement of total [^{3}H]inositol phosphate production, acini were labeled with 20 μCi/ml [^{3}H]inositol for 90 min at 37°C. The labeled acini were washed twice with PSS containing 10 mM LiCl and resuspended in the same solution. Aliquots of acini (0.6 ml) were incubated with agents for 90 s and the incubation terminated by addition of an equal volume of 20% ice-cold trichloroacetic acid. After centrifugation at 1000 × *g* for 15 min, 0.9 ml of each supernatant was washed three times with water-saturated diethylether, neutralized with 1 M $KHCO_3$, and diluted with 2.5 ml of water. Analysis of total [^{3}H]inositol phosphates was carried out by the method described by Berridge [14].

Measurement of [^{125}I]endothelin binding to acini

For the measurement of endothelin binding, 4 ml aliquots of acinar suspension were placed in 25 cm^2 tissue culture flasks, together with 5 pM [^{125}I]ET-1 and specified concentrations of ET. Non-specific binding determined in the presence of 100 nM ET-1 was less than 10% of total binding. Incubations were performed in a shaking water bath at 37°C, and the binding reaction terminated by removing triplicate 1 ml aliquots of acinar cell suspension from each flask and layering each aliquot over 2 ml ice cold 0.9% NaCl. Following centrifugation at 200 × *g* for 3 min, the pellets were washed twice with 0.9% NaCl and the radioactivity associated with the pellet was determined. Competitive inhibition of [^{125}I]ET binding by non-radioactive ET-1 was analysed using a version of the LIGAND non-linear curve fitting program [15].

Measurement of amylase release from acini

For the measurement of amylase release, acini were preincubated for 30 min, washed once and resuspended in fresh, gassed PSS. Aliquots of 1 ml were then distributed into 20 ml blood dilution vials and incubated with agents at 37°C for 5 or 30 min. The incubation was terminated by centrifugation for 15 s in a microfuge, and amylase released into the supernatant was assayed as previously described, using procion yellow starch as the substrate [23,27]. Amylase release was expressed as the percentage of the total amylase in the acini at the beginning of the incubation.

Results

Stimulation with endothelin-1 increases $[Ca^{2+}]_i$

Stimulation of fura-2 loaded acini, with ET-1 at threshold concentrations of between 10 nM and 50 nM resulted after a delay of 2–4 min in the appearance of a slow, sustained oscillating intracellular free calcium signal ($[Ca^{2+}]_i$) (Fig. 1A) with a frequency of one cycle per 2–4 min, somewhat slower than previously reported for CCK induced oscillations in this tissue [12]. The average amplitude of the rise was 357 ± 37 nM and was characterized by repetitive, pulsatile increases in fluorescence, originating and then returning towards basal values (Fig. 1A). At higher concentrations of ET (100 nM or 1 μM), a different pattern was observed, consisting of a single large transient increase, rapidly rising from a basal concentration of 92 ± 11 nM (S.E.) (n = 37) to a peak of 503 ± 29 nM (n = 23) (100 nM), which returned to near basal values within 3 min (Fig. 1C). Stimulation with 1 μM ET increased $[Ca^{2+}]_i$ to a peak of (544 ± 43 nM) (n = 3), a response very similar in amplitude and profile to that observed with stimulation with 100 nM ET. In only 2 of 23 cells was any indication of a sustained increase in $[Ca^{2+}]_i$ observed, such as is seen with stimulation by CCK, carbachol or bombesin [11,12,16]. Furthermore, in 3 experiments performed on a population of cells in a stired cuvette no indication of a sustained phase of the response was seen (data not shown). The return of the signal to basal was more rapid for ET than CCK (time to 75% decrease 55 ± 6 s, and 105 ± 5 s for ET and CCK, respec-

tively). At concentrations of the peptide intermediate between the induction of oscillations and the transient reponse, the $[Ca^{2+}]_i$ signal consisted of a sharp increase in $[Ca^{2+}]_i$ followed by small irregular oscillations ($n = 3$) (Fig. 1B). At concentrations below 10 nM, no effects of endothelin were seen ($n = 8$). In four experiments no effects on $[Ca^{2+}]_i$ of ET-3 stimulation (10 nM–1 μM) were seen.

Stimulation of acini superfused in a Ca^{2+}-free media with 100 nM ET resulted in a transient increase in $[Ca^{2+}]_i$ of similar amplitude (496 ± 47nM, $n = 4$) to that observed in the presence of extracellular Ca^{2+}, indicating that ET predominantly releases Ca^{2+} from an intracellular store (Fig. 1D). Experiments were performed to assess if ET released the same intracellular store as more traditional agonists, such as CCK. After continued stimulation by a high dose of ET, CCK (1 nM) was still capable of evoking a further increase in $[Ca^{2+}]_i$ (Fig. 2a). Conversely, however, after prior stimulation with CCK, ET-1 was ineffective (Fig. 2B). In another series of experiments thapsigargin (TG), which is known to discharge the agonist-sensitive intracellular Ca^{2+} pool [17], was utilised prior to stimulation with either CCK or ET-1. Neither ET nor CCK applied after TG evoked any further increase in $[Ca^{2+}]_i$, (Fig. 2C). These data indicate that the intracellular pool released by ET is a subset of the total stores in the cell. The total agonist releasable store, however, is accessed by CCK.

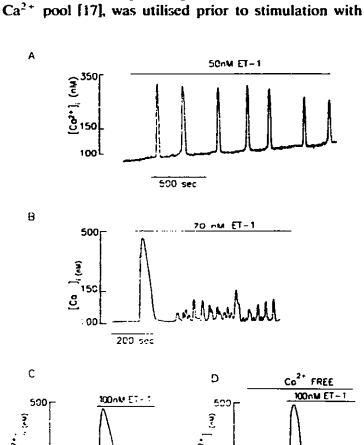

Fig. 1. (A) ET-1 at concentrations between 10 nM–50 nM induced an oscillating $[Ca^{2+}]_i$ signal ($n = 5$). (B) Superfusion of 70 pM ET-1 results in a sharp transient increase in $[Ca^{2+}]_i$, followed by much smaller irregular oscillations. At higher concentrations, (0.1, 1 μM) ET-1 induced a larger, but transient increase in $[Ca^{2+}]_i$ ($n = 16$), which was not significantly different from the response generated in the absence of external Ca^{2+} ($n = 4$) (D).

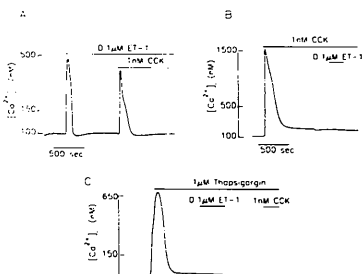

Fig. 2. (A) After stimulation with ET-1 (0.1 μM), subsequent superfusion of CCK (1 nM) is still able to release further Ca^{2+} ($n = 5$); (B) after stimulation with CCK (1 nM), ET-1 (0.1 μM) is unable to release further Ca^{2+} ($n = 3$); (C) after stimulation with thapsigargin (1 μM), neither ET-1 (0.1 μM) or CCK (1 nM) is able to induce any further increase in $[Ca^{2+}]_i$ ($n = 4$).

Endothelin stimulation results in the formation of inositol phosphates

In tissues where an increase in $[Ca^{2+}]_i$ has been described after challenge with ET, it is thought to be mediated at least in part through the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2), which produces inositol 1,4,5-trisphosphate and the subsequent release of Ca^{2+} from intracellular stores [18,19]. As an assay of PIP_2 hydrolysis, acini were labelled with [^{3}H]inositol and total inositol phosphate production was measured. ET-1 was observed to induce PI turnover in a dose dependent manner, however, to a much smaller extent than CCK (Fig. 3). In five experiments ET (1 μM) stimulated PI turnover by 19.3 ± 3.7% of the maximum CCK response. In this series, a maximal concentration of CCK (0.1 μM) increased PI hydrolysis to 1381 ± 424% above basal. In earlier studies 100 pM CCK has been found to be the threshold concentration at which measureable PI hydrolysis can be determined [32].

Receptors for endothelin on rat pancreatic acini

Multiple receptor sub-types have been described for endothelin, which differ in their selectivity for the different members of the endothelin family [20]. We determined whether a specific type of endothelin re-

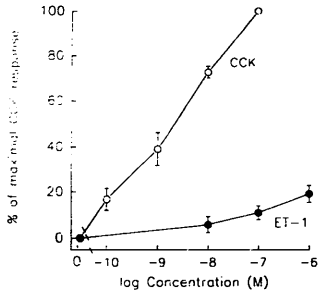

Fig. 3. Concentration dependence of endothelin and cholecystokinin to increase hydrolysis of phosphoinositides in pancreatic acini. Acini pre-labelled with [^{3}H]inositol were incubated for 90 s with either endothelin or CCK and the [^{3}H]inositol phosphates were assayed. Data are the mean ± S.E. of five separate experiments calculated as the % of the maximal CCK response in each particular experiment. Stimulation by 10^{-7} and 10^{-6} M ET-1 was significantly different from basal ($P < 0.05$), using an unpaired t-test. Stimulation by 10^{-8} M ET-1 was significantly different from basal, using ANOVA with the Duncan multiple range test ($P < 0.05$).

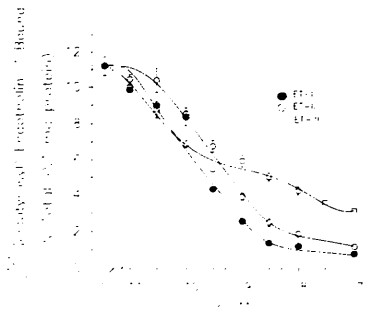

Fig. 4. Competitive inhibition of [^{125}I]ET-1 binding by ET-1, ET-2 and ET-3 in rat pancreatic acini. Acini were incubated and binding assayed as described in Materials and Methods. A representative experiment of three others is shown.

ceptor could be identified on isolated pancreatic acini. Specific binding of [^{125}I]ET-1 to acini was observed which reached a steady state of 9.9 ± 0.8% per 100 ng of acinar cell protein after 120 min. Binding of [^{125}I]ET to rat acini was competitively inhibited by ET-1, ET-2 and ET-3 in a concentration dependent manner (Fig. 4). The competitive inhibition curves for ET-1 and ET-2 were sigmoidal, parallel and inhibition occurred over two orders of magnitude of ET-1 or ET-2 concentrations. ET-1 was approx. 2 times as potent as ET-2 at inhibiting [^{125}I]ET-1 binding (IC_{50} occurring at 160 and 410 pM for ET-1 and ET-2, respectively). The curve describing inhibition by ET-3 was non-parallel and ET-3 at a concentration of 0.1 μM was unable to inhibit binding to non-specific levels. Non-linear analysis of competitive inhibition of [^{125}I]ET-1 binding to rat acini by ET-1 was best fit by a one-site model yielding a K_d of 108 ± 12 pM and B_{max} of 171 ± 17 fmol/mg acinar protein ($n = 3$). These data are consistent with the receptor on acini being a endothelin type 1 receptor, similar in both apparent affinity and rank potency order to that described on rat medullary interstitial cells [20].

Endothelin does not stimulate amylase secretion

Occupation of secretagogue receptors coupled to PI-metabolism in the pancreas (CCK, bombesin, muscarinic cholinergic) invariably leads to the secretion of amylase and, therefore, the ability of endothelin to induce amylase secretion was tested. Surprisingly, in all experiments endothelin (100 pM–1 μM) caused no significant increase in secretion over basal after either 30 min incubation (Table I) or after 5 min (data not shown), whilst in the same experiments CCK (100 pM) and a maximal concentration of thapsigargin (2 μM) induced significant secretion. In another series of experiments ET (100 pM–1 μM) was shown not to effect secretion evoked by a maximal concentration of CCK (100 pM) (data not shown). In addition, when endothelin was added concurrently with the phorbol ester TPA, no potentiation of secretion was observed. Secre-

TABLE I

Effects of endothelin, TPA, TG and CCK on amylase release

Comparision of the effects of ET-1, CCK and TG on amylase secretion from rat pancreatic acini in the presence or absence of phorbol ester. Values are means ± S.E. from 3 experiments, in each of which amylase release was measured over a 30 min period in duplicate.

Amylase release (% of total)	Basal	100 nM Endo	1 μM Endo	2 μM TG	100 pM CCK
without 1 μM TPA	3.7 ± 0.3	4.6 ± 0.7	3.4 ± 0.5	9.8 ± 1.8	30.8 ± 2.6
with 1 μM TPA	14.1 ± 0.94	12.1 ± 1.3	12.1 ± 1.7	25.4 ± 2.8	28.8 ± 2.2

tion induced by TG, however,was significantly increased by TPA (Table I).

Discussion

In this present study we have shown for the first time a direct action of endothelin on rat pancreatic acinar cells, involving activation of the PI-PLC system leading to an increase in $[Ca^{2+}]_i$. Stimulation of pancreatic acinar cells with PI-PLC linked agonists, such as CCK, bombesin or acetylcholine gives rise to distinct patterns of $[Ca^{2+}]_i$ signals depending on the dose of agonist. Characteristically, at low concentrations of agonists, stimulation results in the generation of an oscillatory $[Ca^{2+}]_i$ signal, a response which is primarily due to the release of $[Ca^{2+}]_i$ from intracellular stores [12,21]. In this respect, stimulation with ET results in such a typical response, namely the generation of a pulsatile $[Ca^{2+}]_i$ signal (see Fig. 1A), although the frequency is lower. Higher concentrations of traditional agonists, however, result in a different pattern, which is characterised by a large initial spike, followed by a much lower sustained plateau [16] (see also Fig. 2B), a response which is dependent both on release of Ca^{2+} from intracellular stores and the influx of Ca^{2+} from the extracellular space. In this respect, stimulation with high doses of ET differs from more established agonists as no sustained elevation in the signal was seen. Moreover, the increase in $[Ca^{2+}]_i$ appears to be almost exclusively through a release of Ca^{2+} from intracellular stores, as little difference was observed between cells stimulated in the presence or absence of external $[Ca^{2+}]_i$. The source of this intracellular Ca^{2+} release appears to be the same store which is accessed by CCK. ET, however, is only able to release a subfraction of these stores.

The absence of any measurable amylase secretion in response to ET is somewhat surprising as all previously studied pancreatic PI-linked agonists induce amylase secretion [23]. This lack of secretion may be related to the relatively small $[Ca^{2+}]_i$ signal induced by ET-1 together with the absence of any sustained rise in the signal. This sustained increase in the $[Ca^{2+}]_i$ signal, attributed to Ca^{2+} influx from the extracellular fluid has been shown to be an important modulator of amylase secretion. Recently, Tsunoda and colleagues [24] have shown that experimental manoeuvres designed to perturb Ca^{2+} influx, such as administration of inorganic Ca^{2+}-channel blockers, markedly reduce the sustained increase in $[Ca^{2+}]_i$ and amylase secretion. These observations are consistent with the well established fact that amylase secretion is affected by removal of extracellular Ca^{2+} by chelation with EGTA [25,26]. The observation that TG, a Ca^{2+} mobilising agent which induces sustained Ca^{2+} influx (see Fig. 2C), can stimulate amylase secretion is also consistent with the idea that the inability of ET to induce Ca^{2+} influx is closely related to the lack of any measurable amylase secretion. A similar situation is well established in another model secretory system, the bovine adrenal chromaffin cell, where only agonists which induce Ca^{2+} influx are able to stimulate full secretion of catecholamines [30]. In this system investigators have postulated that Ca^{2+} influx, as opposed to release of intracellular stores, facilitates secretion by increasing $[Ca^{2+}]_i$ at the plasma membrane, where presumably the secretory apparatus is located [31].

The concentrations of endothelin which are effective on rat pancreatic acini are higher than those reported to be efficacious in other tissues. For example, in hepatocytes, ET-1 concentrations of 0.1–1 nM can evoke an increase in $[Ca^{2+}]_i$ [6]. As the apparent affinity of the receptor for endothelin that we report does not greatly differ from that reported in other tissues [6,20], including the hepatocyte, one possible explanation for the high concentrations is that rapid desensitisation or internalization of the receptor may occur, a phenomenon reported for ET in other tissues [22]. This idea is consistent with ET only inducing a small turnover of inositol-phosphates and, consequently, only releasing a portion of stored intracellular Ca^{2+}. The observation that the time for the ET response to decay is quicker than for CCK may also indicate desensitisation of the ET receptor. An alternative explanation is that the pancreatic endothelin receptor may show a very low efficiency for coupling to its transduction mechanism, possibly at the level of the G-proteins and this may result in the production of relatively small amounts of second messengers in comparison with CCK. It is also possible that Ca^{2+} release induced by endothelin is not mediated exclusively by 1,4,5-IP_3. Evidence exists in the exocrine pancreas for an alternate signalling pathway, which results in an increase in $[Ca^{2+}]_i$; this includes reports that at concentrations of traditional agonists which induce Ca^{2+}-oscillations and maximal amylase secretion no changes in PI- hydrolysis can be detected [32,33,34].

This study has shown the existence of endothelin receptors on acinar cells and that in terms of signal transduction ET appears to function in the exocrine pancreas as a PI-coupled agonist. However, some differences exist compared to stimulation with more traditional PI-coupled agonists, the most striking being the lack of any measurable Ca^{2+} influx or accompanying amylase secretion. Pancreatic agonists coupled to PI-turnover have a variety of actions in addition to the secretion of amylase, including modulation of ion-channels, leading to fluid secretion [28] and the stimulation of growth and development of the pancreas [29]. It is possible that further study of the consequences of ET receptor occupation will reveal a role in one of these functions.

Acknowledgements

This work was supported by NIH grants DK-41122 and DK-41225 (JAW), 5T32 DK07367-12 (GTB) the Michigan Gastointestinal Peptide Center (DK34933) and a Marion Merrel Dow fellowship (DiY). We thank Tim Essington for valuable technical assistance on these studies

References

1 Yanagisawa, M., Kurihara, H., Kimura, S., Tombe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. and Masaki, T. (1988) Nature 332, 411–414.
2 Simonson, M.S and Dunn, M.J. (1990) Hypertension 15 (supp I), I-5–I-12.
3 Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K. and Masaki T. (1989) Proc. Natl. Acad. Sci. USA 86, 2863–2867.
4 De Nucci, G., Thomas, R., D'Orleans-Juste, P., Antunes, E., Walder, C., Warner, T.D. and Vane, J.R. (1988) Proc. Natl. Acad. Sci. USA 85, 9797–9800.
5 Koseki, C., Imai, M., Hirata, M. Yanagisama, M. and Masaki, T. (1988) in Abstracts of the International symposium on Biosignalling in Cardiac and Vascular systems 1988, pp. 37.
6 Seradeil-Le Gal, C., Joureaud, C., Sanchez-Bueno, A., Raufaste, D., Roche, B., Preax, A.M., Maffrand, J.P., Cobbold, P.H., Hannoune, J.M. and Lotersztajn, S. (1991) J. Clin. Invest. 87, 133–138.
7 Takuwa, N, Takuwa,Y., Yanagisawa M., Yamashita K. and Masaki, T. (1989) J. Biol. Chem. 264, 7856–7861.
8 Simonson, M.S., Wann, S., Mene, P., Dubyak, G.R., Kester, M., Nakazato, Y. Sedor, J.R. and Dunn, M.J. (1989) J. Clin. Invest. 83, 708–712.
9 Wiklund, N.P., Ohlen, A. and Cederqvist, B. (1988) Acta Physiol. Scand. 134, 311–312.
10 Williams, J.A., Korc, M. and Dormer, R.L. (1978) Am. J. Physiol. 235, E517–E524.
11 Yule, D.I. and Gallacher, D.V. (1988) FEBS Lett. 239, 358–362.
12 Tsunoda, Y., E.L. Stuenkel, and Williams, J.A. (1990) Am.J.Physiol. 258, C147–C155.
13 Grynkiewicz, G., M. Poenie, and Tsien, R.Y. (1985) J. Biol. Chem. 260, 3440–3450.
14 Berridge, M.J. (1983) Biochem. J. 212, 849–858.
15 Munson, P.J. and Rodbard, D. (1980) Anal. Biochem. 107, 220–239.
16 Matosaki, T., Zhu, W-Y., Tsunoda, Y., Goke, B. and Williams, J.A. (1991) Am. J. Physiol. 260, G858–G864.
17 Kwan, C.Y., Takemura, H., Obie, J.F., Thapstrup, O. and Putney, J.W., Jr. (1990) Am. J. Physiol. 249, C1006–C1015.
18 Masaki, T (1989) J. Cardiol. Pharmacol. 13, supp. 5, S1–S4.
19 Berridge, M.J. (1987) Annu. Rev. Biochem. 56, 159–193.
20 Wilkes, B.M., Ruston, A., Mento, P., Giradi, E., Hart, D., Van Der Molen, M., Barnett, R. and Nord, R. (1991) Am. J. Physiol. 260, F579–589.
21 Yule, D.I., Lawrie, A. and Gallacher, D.V. (1991) Cell Calcium 12, 145–151.
22 Arai, H., Hori, S., Arampri, I., Onkubo, H. and Nakanishi, S. (1990) Nature 348, 730–732.
23 Williams, J.A., Korc, M. and Dormer, R.L. (1978) Am. J. Physiol. 253, E517–E528.
24 Tsunoda, Y., Stunkel, E.L. and Williams, J.A. (1990) Am. J. Physiol., G792–G801.
25 Gardener, J.D., Costenbader, C.L. and Uhlemann, E.R. (1979) Am. J. Physiol. 236, E754–E762.
26 Williams, J.A. (1980) Am. J. Physiol. 238, E517–E524.
27 Jung, D.H. (1980) Clin. Chim. Acta 100, 7–11.
28 Petersen, O.H. (1980) The Electrophysiology of Gland Cells, Academic Press, New York.
29 Soloman, T.E. in Physiology of the digestive tract (Johnson, L.R., ed.), pp. 873–893, Raven Press, New York.
30 Cheek, T.R. and Burgoyne, R.D. (1985) Biochim. Biophys. Acta 846, 167–173.
31 Burgoyne, R.D. (1991) Biochim. Biophys. Acta 1071, 174–202.
32 Matozaki, T., Goke, B., Tsunoda, Y., Rodriquez, M., Martinez, J. and Williams, J.A. (1990) J. Biol. Chem. 265, 6247–6254.
33 Rowley, W.H. Sato, S., Huang, S.C. Collado-Escobar, D.M., Beaven, M.A., Wang, Lu-Hua., Martinez J., Gardner, J.D. and Jensen, R.T. (1990) Am. J. Physiol. 259, G655–G665.
34 Saluja, A.K., Powers, R.E. Steer, M.L. (1989) Biochem. Biophys. Res. Comm. 164, 8–13.