MOLECULAR PHARMACOLOGY, 28:100–106

Expression of Both α_1- and α_2-Adrenoceptors in an Insulin-Secreting Cell Line

Parallel Studies of Cytosolic Free Ca^{2+} and Insulin Release

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Received December 31, 1984; Accepted May 29, 1985

SUMMARY

Changes in intracellular free Ca^{2+}, $[Ca^{2+}]_i$, and immunoreactive insulin release in response to α-adrenergic agents were measured in RINm5F cell suspensions. Cells were loaded with the fluorescent indicator quin 2 for monitoring $[Ca^{2+}]_i$.

Epinephrine (1 μM), which inhibited alanine-stimulated insulin release by 73%, evoked a transient rise in $[Ca^{2+}]_i$. This rise is in part due to Ca^{2+} mobilization, since it is still present in the absence of extracellular Ca^{2+}. The α_2-adrenergic agonist clonidine (10 μM) mimicked the epinephrine effect on insulin release without any change in $[Ca^{2+}]_i$. In contrast, the α_1-adrenergic agonist phenylephrine (10 and 100 μM) raised $[Ca^{2+}]_i$, albeit to a lesser extent than epinephrine. Phenylephrine enhanced basal, but had no effect on alanine-stimulated insulin release. To examine further the nature of the effect of epinephrine, specific α-adrenergic blocking agents were employed. The epinephrine-induced increase in $[Ca^{2+}]_i$ could be inhibited by the α_1-adrenergic antagonists BE2254 (0.1 μM) and prazosin (0.01 μM). In the presence of these blockers, epinephrine was still able to inhibit insulin release.

When α_2-adrenergic receptors were blocked by the addition of idazoxan (0.1 and 1 μM), epinephrine still raised $[Ca^{2+}]_i$. At the higher concentration of idazoxan, the epinephrine inhibition of insulin release was completely overcome. The α-adrenergic agonists did not attenuate the alanine-induced rise in $[Ca^{2+}]_i$.

This study shows that both subtypes of α-adrenergic receptors are present in the insulin-secreting cell line RINm5F. The activation of α_1-adrenergic receptors leads to an increase in $[Ca^{2+}]_i$. In contrast, the inhibition of insulin release due to epinephrine is mediated through α_2-adrenergic receptors. The α_2-adrenergic mechanism does not involve changes in $[Ca^{2+}]_i$, but is rather exerted at a later step in the secretory process.

INTRODUCTION

The importance of Ca^{2+} for stimulus-secretion coupling in insulin-secreting cells is widely accepted (1) and has now been demonstrated directly by the use of quin 2 (2–4). Certain secretagogues were thus shown to raise the intracellular free Ca^{2+} concentration, $[Ca^{2+}]_i$.[2] Epinephrine and the sympathetic nervous system are known to exert inhibitory effects on insulin secretion which are of physiological importance (1, 5). Although it is well established that the inhibitory action of epinephrine is mediated by α_2-adrenergic receptors, the underlying mechanism is unknown (6–10). It has been proposed that epinephrine might inhibit insulin release by lowering $[Ca^{2+}]_i$ (11). Further, it was reported that epinephrine attenuates glucose-stimulated $^{45}Ca^{2+}$ uptake by isolated islets (12, 13), an effect not mimicked by the α_2-agonist clonidine, which also inhibits insulin release (7, 9). The difference between the two agonists may be due to epinephrine binding to other classes of adrenergic receptors. Both α_2- and β-adrenergic receptors have been demonstrated directly on pancreatic islet cells (14, 15), whereas the presence of α_1-receptors has only been inferred (16).

The aim of the present study was to investigate whether epinephrine-induced inhibition of insulin release is accompanied by a change in $[Ca^{2+}]_i$. Experiments with quin 2 as an intracellular Ca^{2+} indicator usually require a large amount of cells. Therefore, the insulin-producing cell line RINm5F was used, which has retained the capacity to release insulin in response to a

This work was supported by Grant 3.246-0.82 SR from the Swiss National Science Foundation.

[1] This study is part of the Ph.D thesis of S.U.

[2] The abbreviations used are: $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

0026-895X/85/020100-07$02.00/0

variety of secretagogues (2, 17). This cell line expresses several hormone receptors (18) including a receptor which mediates epinephrine inhibition of insulin release (17). Alanine, which raises $[Ca^{2+}]_i$, was used as an insulin secretagogue in combination with various adrenergic agonists and antagonists. The results presented here demonstrate that α_2-adrenergic inhibition of insulin release is not mediated by changes in $[Ca^{2+}]_i$. An unexpected rise in $[Ca^{2+}]_i$ was induced by epinephrine which was shown to be mediated by α_1-receptors.

EXPERIMENTAL PROCEDURES

Cell culture. RINm5F cells were cultured as described elsewhere (2, 17). Cell suspensions were prepared 3 h before the experiment by detaching the cells from the culture flasks with trypsin (0.025%) in Ca^{2+}/Mg^{2+}-free 0.9% NaCl solution supplemented with 0.27 mM EDTA. The cells were resuspended in RPMI 1640 supplemented with 10 mM Hepes, 1% newborn calf serum, and antibiotics (2) and maintained in a spinner flask at a concentration of 0.5–0.6 × 10^6 cells/ml.

Insulin release. The cells were washed twice in serum-free RPMI 1640 containing 25 mM Hepes, pH 7.4, by centrifugation at 150 × *g* for 10 min and resuspended in 32–35 ml of Krebs-Ringer bicarbonate Hepes buffer containing 25 mM Hepes, 5 mM $NaHCO_3$, 1.1 mM $CaCl_2$, 0.1 mM EGTA, 2.8 mM glucose, and 0.07% bovine serum albumin. Aliquots of the cell suspension were distributed into siliconized glass tubes by diluting the cells 1:2 to a final volume of 1 ml/tube. The cell concentration in the tubes was 0.66 ± 0.04 × 10^6 cells/ml (n = 11), and the insulin content 230 ± 37 ng insulin/10^6 cells (n = 8). The cells were preincubated for 15 min at 37° and thereafter centrifuged at 150 *g* for 5 min at room temperature. 800 μl of the supernatant was removed and stored at −20°. After addition of 800 μl of buffer containing the test substances at appropriate final concentrations, the cells were incubated for 10 min at 37°. The incubation was stopped by cooling the tubes on ice. The tubes were centrifuged for 10 min at 150 × *g*, the supernatant was removed, and the cellular insulin content was extracted by addition of 1 ml of acid ethanol (ethanol/water/concentrated HCl, 140:57:3 by volume).

All samples were stored at −20° until measurement of insulin by radioimmunoassay (19). The insulin released during the incubation was estimated after correction for the hormone release during the preincubation.

Measurement of cytosolic free Ca^{2+}. The cells were washed twice and usually resuspended in 1 ml of serum-free RPMI 1640 supplemented with 25 mM Hepes and 0.5% bovine serum albumin, pH 7.4 at cell concentrations of 20–30 × 10^6/cells/ml. After a 10-min preincubation at 37°, quin 2 acetoxymethyl ester (1 mM stock solution in dimethyl sulfoxide) was added to one aliquot of the cells at a final concentration of 100 μM. Another aliquot of the cells (controls) received dimethyl sulfoxide at a final concentration of 1%. After 10 min at 37°, the suspensions were diluted with 4 volumes of RPMI 1640 and the incubation was continued for another 50 min. Thereafter the cells were washed, resuspended in RPMI 1640, and kept at 18–20° until use.

Aliquots of the cell suspension were centrifuged (5 min at 150 × *g*) and resuspended in 2 ml of Krebs-Ringer bicarbonate Hepes buffer with the same composition as above but without albumin in a glass cuvette. The fluorescence was measured at 37° under continuous stirring using an excitation wavelength of 339 nm and an emission wavelength of 492 nm (Perkin-Elmer, LS-3 fluorometer). The calibration of each trace is described elsewhere(2). For the calculations of intracellular Ca^{2+} concentrations, a K_D of 115 nM was used in Equation 1 of Tsien *et al.* (20). The cell concentration in the cuvette was 1.51 ± 0.17 × 10^6 cells/ml (n = 12). The intracellular quin 2 concentration measured was 1.22 ± 0.14 mM (n = 11). For the calibration of $[Ca^{2+}]_i$ and to examine for nonspecific interference of the test substances, nonloaded control cells were always treated in parallel.

Results are expressed as mean ± SE. Statistical analysis was by Student's t-test for unpaired data, except for the changes in $[Ca^{2+}]_i$ which were evaluated by paired analysis.

Materials. The materials and their sources were as described elsewhere (2, 10) except for: quin 2 acetoxymethyl ester, the Radiochemical Centre, Amersham (Bucks, United Kingdom); phenylephrine, Sigma; idazoxan (RX 781094, 2-(2-1,4-benzodioxanyl))2-imidazoline HCl), a generous gift from Dr. M. D. Day (Reckitt and Colman, Pharmaceutical Division, Kingston-upon-Hull, United Kingdom); BE2254 (2-[β-(4-hydroxyphenyl)ethylaminomethyl]-tetralone), provided by Beiersdorf A.G. (Hamburg, Federal Republic of Germany); prazosin hydrochloride, provided by Pfizer A.G. (Zürich, Switzerland).

RESULTS

As shown in Fig. 1, epinephrine (1 μM) and clonidine (10 μM), an α_2-adrenergic agonist, had no effect on basal insulin release in RINm5F cell suspensions. The α_1-adrenergic agonist phenylephrine caused a slight increase of basal insulin release at 10 μM ($p > 0.05$) and a more marked rise at 100 μM ($p < 0.001$). Alanine (10 mM) stimulated insulin release 2.6-fold over the 10-min incubation period. This stimulation was inhibited by epinephrine by 73% ($p < 0.001$). Clonidine inhibited alanine-stimulated insulin release by 54% ($p < 0.001$). Phenylephrine at 10 μM and 100 μM did not change alanine-stimulated insulin release significantly.

Specific blockers for α_1- and α_2-adrenoceptors were used to examine the nature of the epinephrine effect (Table 1). The new α_2-adrenergic antagonist idazoxan (21, 22) completely overcame the epinephrine-induced inhibition of alanine-stimulated insulin release at concentrations of 1, 3, and 10 μM. At 0.1 μM, idazoxan still blocked the epinephrine effect by 57%. At 0.1 μM, the commonly used α_2-adrenergic antagonist α-yohimbine did not attenuate the epinephrine-induced inhibition significantly ($p > 0.4$). At 0.3 μM, it blocked the epinephrine effect by 47%, and at 1 μM α-yohimbine, the epinephrine action was completely antagonized. Surprisingly, the α_1-antagonist BE2254 (23, 24) showed similar effects as α-yohimbine. At 0.1 μM, BE2254 attenuated the inhibition of insulin release by 21% ($p < 0.02$). In the presence of 0.3 μM BE2254, epinephrine still in-

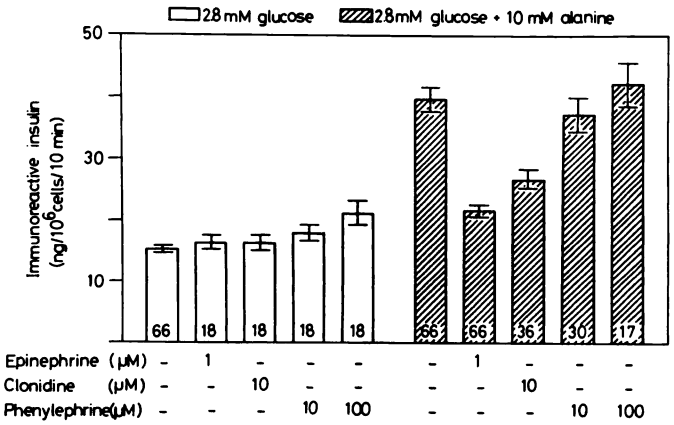

FIG. 1. *Effect of alanine and adrenergic agonists on insulin release from RINm5F cells*

Cell suspensions were incubated at 37° for 10 min as described under "Experimental Procedures." Alanine, epinephrine, clonidine, and phenylephrine were added as indicated. Values are mean ± SE of the indicated number of observations from 11 separate experiments.

TABLE 1

Effect of α-adrenergic antagonists on the epinephrine-induced inhibition of alanine-stimulated insulin release

Insulin release was measured after 10 min of incubation at 37° (see "Experimental Procedures"). In each experiment, the remaining epinephrine inhibition in the presence of α-adrenergic antagonists was calculated from the ratio of insulin release in the presence and absence of antagonists. The inhibition of alanine (10 mM)-stimulated insulin release by epinephrine (1 μM) was set to 100%. Results are given as mean ± SE; numbers of observations are given in parentheses. ND, not determined.

Antagonist	Epinephrine inhibition at antagonist concentration					
	0	10 μM	3 μM	1 μM	0.3 μM	0.1 μM
				%		
Idazoxan	100 ± 12 (18)	−7 ± 6 (18)	−29 ± 8 (12)	−1 ± 9 (18)	ND	43 ± 12 (18)
α-Yohimbine	100 ± 8 (6)	ND	ND	16 ± 10 (6)	53 ± 9 (6)	90 ± 11 (6)
BE2254	100 ± 6 (18)	ND	ND	10 ± 12 (12)	60 ± 7 (18)	79 ± 6 (18)
Prazosin	100 ± 8 (18)	59 ± 9 (6)	ND	100 ± 10 (18)	ND	103 ± 8 (18)

hibited insulin release by 60%, while 1 μM antagonist completely blocked the epinephrine effect. In contrast, the widely used α_1-adrenergic antagonist prazosin was more specific. Prazosin did not interfere with the epinephrine action on alanine-stimulated insulin release at concentrations of 1, 0.1 (Table 1),and 0.01 μM (data not shown). At 10 μM, prazosin blocked the epinephrine effect on insulin release by 41% ($p < 0.02$, Table 1). Taken together, these data confirm previous conclusions that epinephrine inhibits insulin release by activation of α_2-adrenergic receptors.

It is of interest that idazoxan at 3 μM not only completely overcame the epinephrine-induced inhibition of alanine-stimulated insulin release but also revealed an enhancing effect of epinephrine. The value of −29% in Table 1 denotes an enhancement of alanine-stimulated insulin release by the same percentage. This stimulation was significantly different from the data obtained both with 1 and 10 μM idazoxan ($p < 0.005$).

In parallel, cells were loaded with the fluorescent indicator quin 2 for the measurement of $[Ca^{2+}]_i$. We have shown previously that the insulin release response to stimulators is comparable in quin 2-loaded and control cells (2). Likewise, epinephrine is still capable of inhibiting stimulated insulin release in quin 2-loaded cells (data not shown). In Fig. 2 are shown representative traces of quin 2 fluorescence in the presence of alanine and adrenergic agonists, and the pooled results are given in Table 2. The average basal intracellular Ca^{2+} concentration was 120 ± 8 nM. Epinephrine (1 μM) raised $[Ca^{2+}]_i$ 1.9-fold (Fig. 2A and Table 2A). The rise was transient and of about 1-min duration. At 10 μM epinephrine, the rise in $[Ca^{2+}]_i$ was not significantly different ($p > 0.2$, Table 2B). In contrast, epinephrine had no effect on membrane potential when measured with the fluorescent probe bisoxonol (data not shown). Alanine (10 mM), which depolarizes the cells (2), increased $[Ca^{2+}]_i$ 3.2-fold (Fig. 2B and Table 2G). This increase could be reversed by the Ca^{2+} channel blocker nifedipine (Fig. 2A) in a similar way as shown recently with verapamil (2, 4). Alanine still raised $[Ca^{2+}]_i$ 3-fold when added 2 min after epinephrine (Fig. 2A and Table 2H). Moreover, when epinephrine was added on top of alanine, a further and transient rise in $[Ca^{2+}]_i$ ensued (Fig. 2B). In four separate experiments with this protocol, alanine raised $[Ca^{2+}]_i$ by 283 ± 95 nM and epinephrine by 81 ± 8 nM. As can be

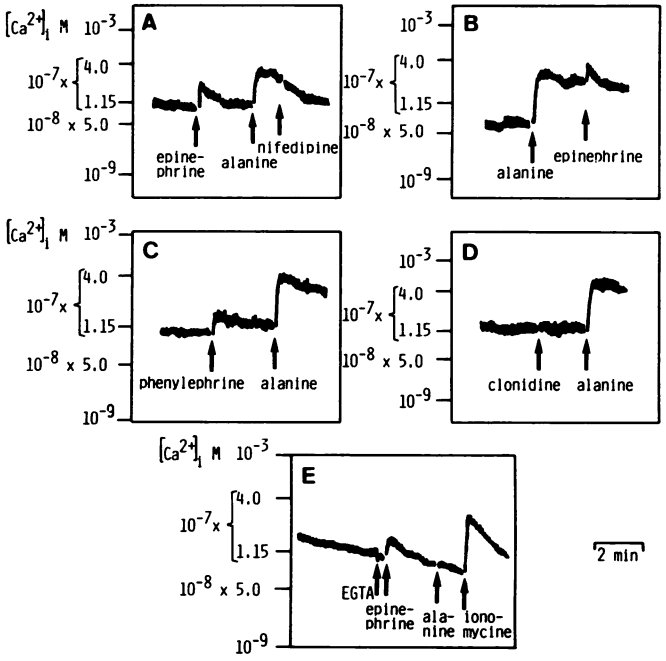

FIG. 2. *Effect of adrenergic agonists and alanine on $[Ca^{2+}]_i$ in RINm5F cells*

Traces A–D, cells were resuspended in Krebs-Ringer bicarbonate Hepes buffer containing 1.1 mM Ca^{2+} and 0.1 mM EGTA; *trace E*, cells were resuspended in Ca^{2+}-free buffer (see "Experimental Procedures"). The final concentrations of test substances added were: epinephrine, 1 μM; phenylephrine, 10 μM; clonidine, 10 μM; alanine, 10 mM; ionomycin, 1 μM; and nifedipine, 1 μM.

seen from Fig. 2C, phenylephrine (10 μM) mimicked the effect of epinephrine, albeit to a limited extent. Phenylephrine was more potent at 100 μM than at 10 μM (Table 2, C and D). Phenylephrine also enhanced $[Ca^{2+}]_i$ already raised by alanine (not shown). In contrast, clonidine (10 μM) did not change $[Ca^{2+}]_i$ (Fig. 2D and Table 2E). The alanine-induced rise in $[Ca^{2+}]_i$ was neither affected by clonidine nor by phenylephrine when the amino acid was added after the α-adrenergic agents (Fig. 2, C and D, and Table 2, I and J). Higher concentrations of alanine did not raise $[Ca^{2+}]_i$ further (data not shown).

The preceding experiments were carried out in the presence of about 1 mM external Ca^{2+}. To investigate whether epinephrine is able to mobilize Ca^{2+} from internal stores, experiments were performed using Ca^{2+}-depleted buffer. To this end, cells were resuspended in

TABLE 2

Effect of adrenergic agonists and alanine on cytosolic free Ca^{2+}

The experiments were carried out with the same protocol as that in Fig. 2. Control values represent $[Ca^{2+}]_i$ immediately preceding the addition of the test substances. In the experiments given in lines H, I, J, alanine was added approximately 2 min after the agonists, and control refers to the steady state $[Ca^{2+}]_i$ prior to alanine addition. The results are mean ± SE for the indicated number of traces. Δ is the maximal difference evoked by the test substances. Statistical analysis was by Student's *t*-test for paired data.

Test substances	$[Ca^{2+}]_i$			*n*	*p*
	Control	Test	Δ		
		nM			
A 1 μM epinephrine	120 ± 8	225 ± 18	105 ± 12	23	<0.001
B 10 μM epinephrine	95 ± 9	168 ± 23	73 ± 15	7	<0.005
C 10 μM phenylephrine	106 ± 18	128 ± 18	22 ± 6	5	<0.025
D 100 μM phenylephrine	107 ± 13	161 ± 17	54 ± 5	8	<0.001
E 10 μM clonidine	118 ± 19	111 ± 15	−7 ± 6	4	>0.3
F 1 μM epinephrine in Ca^{2+}-free medium	100 ± 11	148 ± 19	48 ± 8	4	<0.01
G 10 mM alanine	109 ± 14	348 ± 80	275 ± 74	5	<0.001
H 10 mM alanine after 1 μM epinephrine	148 ± 12	446 ± 51	298 ± 44	16	<0.001
I 10 mM alanine after 10 μM phenylephrine	129 ± 16	399 ± 90	270 ± 92	4	<0.001
J 10 mM alanine after 10 μM clonidine	114	501	387	2	

Ca^{2+}-free Krebs-Ringer bicarbonate Hepes buffer from which Ca^{2+} and EGTA had been omitted. The free Ca^{2+} concentration of this buffer is approximately 10 μM. To lower the Ca^{2+} concentration further, EGTA was added to yield a final chelator concentration of 1 mM. This addition, performed 20 sec prior to the exposure of the cells to epinephrine, results in a free Ca^{2+} concentration of less than 10 nM. Under these conditions, epinephrine was still able to raise $[Ca^{2+}]_i$ (Fig. 2E). However, the increase was only 46% of that seen in the presence of 1 mM Ca^{2+} (Table 2F). As expected, alanine failed to raise $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. Ionomycin, a Ca^{2+} ionophore, which also mobilizes Ca^{2+} from internal stores, raised $[Ca^{2+}]_i$ (Fig. 2E).

The effects of α-adrenergic blockers on the epinephrine-induced rise of $[Ca^{2+}]_i$ are shown in Fig. 3 and Table 3. Idazoxan, tested at three different concentrations, *i.e.*, 0.1, 1, and 10 μM, affected the epinephrine effect in a dose-dependent manner. At the lowest concentration, idazoxan did not change the epinephrine-induced rise of $[Ca^{2+}]_i$. Idazoxan at 1 μM attenuated the increase by 30% ($p > 0.1$) and at 10 μM by 66% ($p < 0.05$) (Fig. 3, A–C, and Table 3, A–D). In the presence of 1 μM α-yohimbine, the epinephrine action was reduced by 53% (Fig. 3D and Table 3E). In contrast to the α_2-adrenergic blocking agents, the α_1-antagonist BE2254 at the low concentration of 0.1 μM abolished the epinephrine-induced rise in $[Ca^{2+}]_i$ (Fig. 3E and Table 3F). The affinity of BE2254 for the α_1-receptors is extremely high. Thus, the antagonist was able to counteract the epinephrine effect even at 0.01 μM, a concentration 100 times lower than that of the agonist (data not shown). Prazosin (0.01 μM) (Fig. 3F and Table 3G) blocked the epinephrine-induced rise of $[Ca^{2+}]_i$ by 88%. It was not possible to use higher concentration of prazosin, since the drug fluoresces strongly at the wavelength used for quin 2 measurements. As already seen with the adrenergic agonists, the alanine-induced rise of $[Ca^{2+}]_i$ was not modified by the antagonists (Fig. 3).

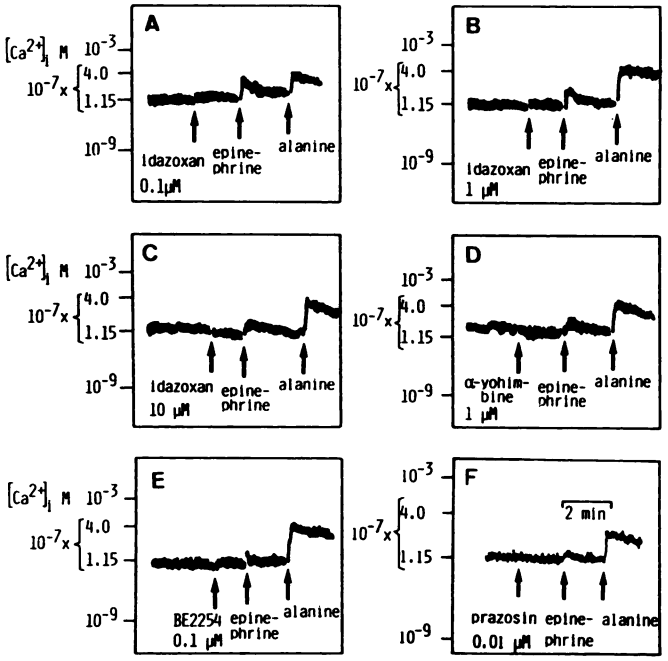

FIG. 3. *Effect of α-adrenergic antagonists on epinephrine- and alanine-induced rises in $[Ca^{2+}]_i$*

The blockers were added as indicated at the appropriate final concentration in the cuvette. The final concentration of epinephrine was 1 μM and that of alanine was 10 mM.

Quin 2 acts not only as a Ca^{2+} indicator, but also as a Ca^{2+} chelator (20, 25, 26). It would be expected that the elevation of $[Ca^{2+}]_i$ caused by Ca^{2+}-mobilizing agents could be attenuated in cells containing high quin 2 concentrations. This is indeed the case for epinephrine, as can be seen in Fig. 4. The rise in $[Ca^{2+}]_i$ induced by epinephrine was correlated inversely to the intracellular

TABLE 3

Effect of α-adrenergic antagonists on epinephrine-induced rise in $[Ca^{2+}]_i$

The experiments were carried out with the same protocol as that in Fig. 3. Basal values represent $[Ca^{2+}]_i$ prior to epinephrine addition. Δ denotes the maximal effect of epinephrine on $[Ca^{2+}]_i$ in the presence and absence of antagonists. Results are given as means ± SE for the indicated number of traces. Statistical analysis was by Student's *t*-test for paired data.

Conditions	$[Ca^{2+}]_i$			*n*	*p*
	Basal	Test (epinephrine, 1 μM)	Δ		
		nM			
A control	124 ± 7	236 ± 17	112 ± 12	26	<0.001
B 0.1 μM idazoxan	130 ± 11	242 ± 26	111 ± 17	5	<0.005
C 1 μM idazoxan	127 ± 10	206 ± 16	79 ± 8	8	<0.001
D 10 μM idazoxan	104 ± 18	141 ± 25	37 ± 7	3	<0.05
E 1 μM α-yohimbine	115 ± 22	168 ± 30	53 ± 8	4	<0.01
F 0.1 μM BE2254	122 ± 15	132 ± 15	10 ± 5	5	>0.1
G 0.01 μM prazosin	91 ± 11	104 ± 11	13 ± 3	7	>0.005

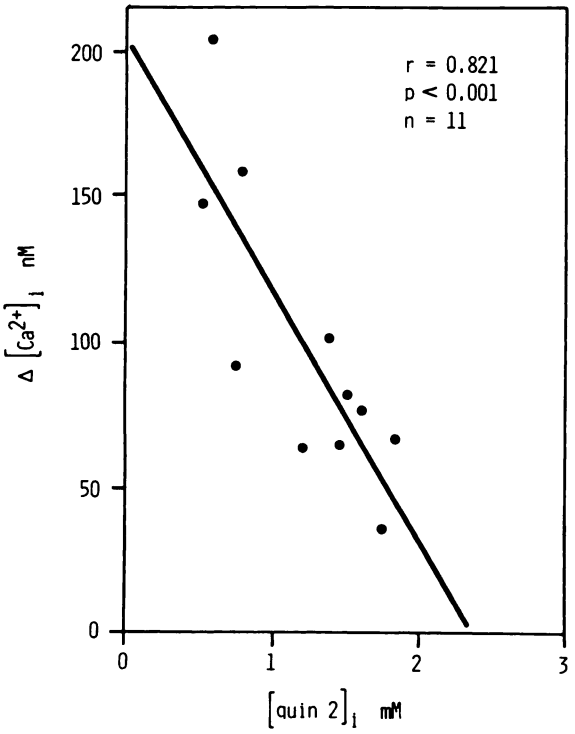

FIG. 4. *Relationship between intracellular quin 2 concentration and epinephrine-induced rise in $[Ca^{2+}]_i$*

The intracellular quin 2 concentration was calculated by comparing the fluorescence of a known amount of quin 2 tetracarboxylate added to a lysate of control cells with intracellular quin 2 in each experiment. An intracellular waterspace of 250 nl/10^6 cells was used for the calculation (see also Ref. 2). Linear regression analysis was applied to values from 11 separate experiments.

quin 2 concentration ($r = -0.82$, $p < 0.001$, $n = 11$). When the linear correlation analysis was applied to the results obtained with alanine, the increase in $[Ca^{2+}]_i$ did not correlate significantly with cellular quin 2 concentration ($r = -0.58$, $p > 0.05$, $n = 11$; data not shown). Similarly, there was no correlation between basal $[Ca^{2+}]_i$ and quin 2 loading ($r = 0.015$, $n = 11$).

DISCUSSION

The model system for studying the regulation of insulin secretion, the RINm5F cell line, has been characterized previously (17). Although this cell line is still sensitive to epinephrine, it should be noted that a 10-fold higher concentration of the agonist is necessary to obtain similar inhibition of stimulated insulin release as that seen in freshly isolated or cultured rat pancreatic islets (10, 13). As is the case in pancreatic islets (6–10), the inhibition of insulin release is mediated by α_2-adrenergic receptors. Thus, the epinephrine effect was mimicked by the α_2-agonist clonidine, but not by the α_1-agonist phenylephrine. Likewise, the α-adrenergic antagonists counteracted the inhibition of insulin release in the following order of potency: idazoxan > α-yohimbine > BE2254 > prazosin.

Under the conditions where epinephrine decreased insulin release, a rapid and transient increase in $[Ca^{2+}]_i$ occurred, which was seen both at basal and alanine-enhanced $[Ca^{2+}]_i$. This effect was not reproduced by clonidine, but mimicked qualitatively by phenylephrine. That activation of α_1-adrenergic receptors indeed mediates the rise in $[Ca^{2+}]_i$ was clearly demonstrated by the use of specific α-adrenergic antagonists. The postsynaptic α_1-antagonists BE2254 (23, 24) and prazosin blocked the epinephrine-induced rise in $[Ca^{2+}]_i$ even at 0.01 μM, attesting to their high affinities for α_1-receptors. In contrast, the α_2-antagonists idazoxan and α-yohimbine only attenuated the $[Ca^{2+}]_i$ rise at concentrations higher than 1 μM. For instance, idazoxan (1 μM), which abolished the epinephrine inhibition of insulin release, lowered the hormone effect on $[Ca^{2+}]_i$ only by 29%. This new α_2-antagonist (21, 22) thus proved more selective than α-yohimbine when considering the effects of the antagonists on $[Ca^{2+}]_i$. Conversely, while prazosin up to 1 μM did not affect the epinephrine-mediated inhibition of insulin release, BE2254 was less specific (Table 1), showing a similar dose response as α-yohimbine. Nonetheless the results demonstrate that the inhibitory effect of epinephrine on insulin release can be completely dissociated from the rise of $[Ca^{2+}]_i$.

The finding that epinephrine raises $[Ca^{2+}]_i$ in insulin secreting cells may be surprising at first sight. In general, an increase in $[Ca^{2+}]_i$ should result in stimulation of insulin secretion (2). This was only clearly seen with 100 μM phenylephrine, which enhanced basal, but not ala-

nine-stimulated insulin release. In the latter situation, phenylephrine still raised $[Ca^{2+}]_i$, which may suggest that the effect of $[Ca^{2+}]_i$ on secretion was already maximal with alanine alone. When epinephrine was used, a slight stimulation of alanine-induced insulin secretion was seen only in the presence of a given idazoxan concentration. The reason for the difference between phenylephrine and epinephrine is not clear.

There are mainly two ways in which $[Ca^{2+}]_i$ is raised: 1) by increasing the passive influx of Ca^{2+} across the plasma membrane, or 2) by mobilization of Ca^{2+} from intracellular stores. As epinephrine was able to raise $[Ca^{2+}]_i$ at least in part even in Ca^{2+}-free medium, the Ca^{2+} seems to originate mainly from intracellular stores. This was also found in hepatocytes, where a rise in $[Ca^{2+}]_i$ has been demonstrated following activation of α_1-receptors by norepinephrine (27, 28). The underlying mechanism seems to involve the breakdown of phosphatidylinositol 4,5-bisphosphate in the cell membrane and the generation of the water-soluble inositol 1,4,5-trisphosphate which elicits Ca^{2+} mobilization from the endoplasmic reticulum (for review, see Refs. 29 and 30).

An interesting application of the quin 2 method is its use not only as a Ca^{2+} indicator but also as an intracellular Ca^{2+} buffer. This approach was used in lymphocytes and human neutrophils to demonstrate the role of Ca^{2+} mobilization in the activation of these cells (25, 26). In the present study, the effect of epinephrine on $[Ca^{2+}]_i$ was inversely correlated to the amount of quin 2 trapped in the cells, *i.e.* the higher the quin 2 loading, the smaller the increase in $[Ca^{2+}]_i$. This is an expected finding for a Ca^{2+}-mobilizing agonist, since an increase of the cytosolic Ca^{2+}-buffering capacity should affect Ca^{2+} mobilization more than Ca^{2+} influx. Indeed, in the same experiments, the rise in $[Ca^{2+}]_i$ due to alanine, which causes Ca^{2+} influx (2, 4), was not affected by quin 2 loading. That alanine causes Ca^{2+} influx was again demonstrated by the finding that nifedipine (Fig. 2A) inhibited Ca^{2+} elevation and that alanine was ineffective in Ca^{2+}-free medium (Fig. 2E). Thus, epinephrine and alanine raise $[Ca^{2+}]_i$ by different mechanisms as also demonstrated by the apparent additivity of the two substances. Furthermore, while alanine depolarizes the cells, epinephrine (and norepinephrine) have been reported to cause a transient hyperpolarization of the β-cell membrane potential (31, 32). This could be due to the transient rise in $[Ca^{2+}]_i$ acting on Ca^{2+}-sensitive K^+ channels in the membrane.

The mode of α_1-adrenergic action is clarified in large part. With respect to α_2-action, the situation is completely different. The results presented here clearly demonstrate that changes in $[Ca^{2+}]_i$ are not involved in the α_2-adrenergic inhibition of insulin release, since the inhibition of alanine-stimulated insulin release was not associated with a decrease in alanine-raised $[Ca^{2+}]_i$. It must be concluded that the α_2-adrenergic action is exerted at a step beyond the increase in $[Ca^{2+}]_i$. We have recently reported that lowering of cellular cyclic AMP levels cannot explain the epinephrine α_2-effect (10). Thus, the situation is similar in β-cells and platelets where neither changes in the concentration of cyclic AMP nor alterations in Ca^{2+} handling appear to be involved in the mode of action of α_2-adrenergic agonists (33). The underlying mechanism remains obscure.

ACKNOWLEDGMENTS

We are greatly indebted to Dr. Benigna Blondel and Mrs. Anne-Sophie Annen for providing the cultured cells.

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