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# Adrenaline inhibition of insulin release: role of the repolarization of the B cell membrane

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**Abstract.** Activation of $\alpha_2$-adrenergic receptors affects several signalling pathways in pancreatic B cells. However, since adrenaline can inhibit insulin release by interfering with a late step of the secretory process, the functional significance of the earlier effects is unclear. In this study, normal mouse islets were used to determine whether the repolarization of the B cell membrane caused by adrenaline contributes to the inhibition of insulin release. The decrease in $^{86}Rb$ efflux and the repolarization of the B cell membrane produced by adrenaline were attenuated by tolbutamide, which depolarizes by blocking ATP-sensitive $K^+$ channels, and by arginine, which depolarizes because of its transport in a charged form. It is also known that adrenaline does not affect the membrane potential and $^{86}Rb$ efflux in B cells depolarized by high $K^+$. These three depolarizing conditions similarly shifted to the right the concentration dependence of adrenaline inhibition of insulin release: the effect of 1 nM and 10 nM adrenaline was reduced, but high concentrations of adrenaline still inhibited insulin release nearly completely under all conditions. In contrast, increasing insulin release by cytochalasin B did not alter the inhibitory potency of adrenaline. It is concluded that the repolarization of the B cell membrane and the ensuing decrease in $Ca^{2+}$ influx play a significant role in the inhibition of insulin release by low concentrations of adrenaline. When high concentrations are used, a more distal effect becomes predominant.

**Key words:** Adrenaline – Adrenoceptors – Insulin release – Pancreatic islets – Ionic fluxes – B cell membrane potential

## Introduction

It is well established that catecholamines inhibit insulin release by activating $\alpha_2$-adrenergic receptors in B cells [27, 34], but there remains controversy about the biochemical events that are involved.

Activation of the receptors decreases adenylate cyclase activity in islet homogenates [19] and lowers cyclic AMP levels in whole islets [30] or purified B cells [33]. However, several studies have established that this mechanism cannot fully account for the inhibition of insulin release [7, 23]. Catecholamines also lower the concentration of cytoplasmic free $Ca^{2+}$ in B cells [1, 28]. This effect is secondary to a partial repolarization of the plasma membrane [6, 8, 28, 32] and the subsequent decrease in $Ca^{2+}$ influx [2, 39, 40]. The repolarization appears to be due to an increase in $K^+$ permeability of the B cell membrane [8, 28] caused by the opening of $K^+$ channels distinct from the ATP-sensitive $K^+$ channels [31].

There is no doubt, however, that catecholamines can inhibit insulin release by a mechanism independent of changes in B cell membrane potential [8] and subsequent to the rise in cytoplasmic $Ca^{2+}$ [28]. The existence of such a mechanism was directly demonstrated by studies [21, 35, 38] using permeabilized islet or RINm5F cells, in which there is no transmembrane electrical potential and in which the cytosolic composition can be controlled. In this model, high concentrations of catecholamines were able to inhibit $Ca^{2+}$- and cyclic-AMP-induced insulin release. $\alpha_2$-Adrenergic agonists can thus interfere with very late steps of the secretory process.

A crucial question is whether this very late effect entirely explains the inhibition of insulin release, the earlier effects being mere epiphenomena, or whether these earlier effects also play a role under physiological conditions. The present study was an attempt to determine whether the repolarization of the B cell membrane brought about by adrenaline contributes to the inhibition of insulin release. If this is the case, the inhibition of release should be attenuated by experimental conditions able to depolarize the B cell membrane and thus to oppose the repolarizing action of adrenaline.

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## Materials and methods

All experiments were performed with islets of fed female NMRI mice (25–30 g), killed by decapitation. For electrophysiological experiments, a piece of pancreas was fixed in a perifusion chamber and islets were partially microdissected by hand. The membrane potential of single B cells was continuously recorded with high-resistance microelectrodes [25]. B cells were identified by the electrical activity they display in the presence of 15 mM glucose.

For all other experiments, islets were isolated after collagenase digestion of the pancreas. After isolation, the islets were loaded with $^{86}Rb$ (used as tracer for K), washed and placed in perifusion chambers [16]. The radioactivity lost by the islets was measured in effluent fractions collected at 2-min intervals. A portion of each effluent fraction was taken for insulin assay. Insulin release was also measured in incubation experiments. Batches of three islets were incubated for 60 min in 1 ml medium containing appropriate concentrations of adrenaline and test substances. A portion of the medium was withdrawn and appropriately diluted at the end of the incubation. Insulin was measured by a double-antibody radioimmunoassay using rat insulin as standard (Novo Research Institute, Bagsvaerd, Denmark).

The medium used was a bicarbonate-buffered solution that contained 120 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$ and 24 mM $NaHCO_3$. It was gassed with $O_2/CO_2$ (47:3), had a pH of 7.4 at 37°C, and, except in electrophysiological experiments, was supplemented with bovine serum albumin (1 mg/ml). When the concentration of KCl was increased, that of NaCl was decreased correspondingly. Oxidation of adrenaline was prevented by 0.5 mM ascorbic acid, which was also added to control solutions.

Adrenaline was obtained from Parke-Davis (Detroit, Mich., USA), tetraethylammonium chloride (TEA) from Fluka (Buchs, Switzerland), tolbutamide from Hoechst A.G. (Frankfurt, FRG), diazoxide was a gift of Schering Corp. (Bloomfield, N.J., USA) and cytochalasin B was kindly provided by ICI Pharma (Destelbergen, Belgium). $^{86}RbCl$ was obtained from the Radiochemical Center (Amersham, Bucks, UK). All other chemicals were obtained from Merck A.G. (Darmstadt, FRG).

Electrophysiological experiments are illustrated by recordings that are representative of the indicated number of experiments performed with different mice, or are presented as means ± SEM. All other data are given as means ± SEM for a certain number of experiments (different islet preparations) or batches of islets. In incubation experiments, insulin release measured in the presence of various concentrations of adrenaline was expressed as a percentage of the control release without adrenaline. The concentration/response curves were then linearized by the least-squares method after probit transformation of the percentages [14]. The concentration of adrenaline producing 50% inhibition ($EC_{50}$) was then obtained from the regression plot.

The statistical significance of differences between means was assessed by comparing control and test conditions by a paired *t*-test, or by an analysis of variance followed by a test of Dunnett [11]. Differences were considered significant when $P$ was lower than 0.05.

## Results

### *Effects of adrenaline in the presence of 15 mM glucose*

Adrenaline caused a rapid biphasic inhibition of $^{86}Rb$ efflux and insulin release from mouse islets perifused with a medium containing 15 mM glucose (Fig. 1). Between 64 min and 70 min, the percentage inhibition of insulin release averaged 56 ± 5%. Adrenaline also altered the repetitive electrical activity recorded in B cells (Fig. 2A). A rapid but transient hyperpolarization with suppression of electrical activity occurred on addition of adrenaline.

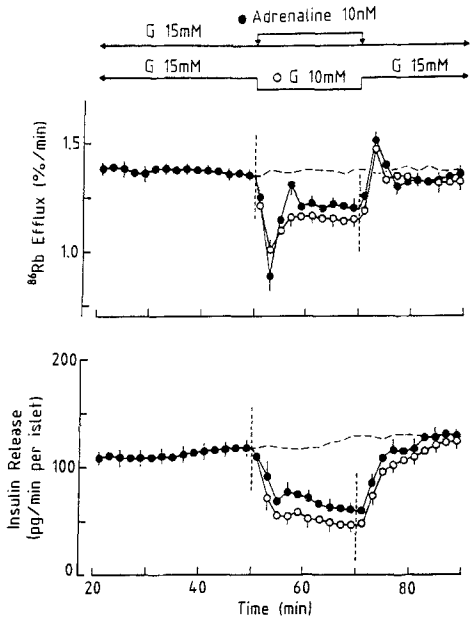


**Fig. 1.** Effects of adrenaline and of a change in the concentration of glucose on $^{86}Rb$ efflux and insulin release from perifused mouse islets. The perifusion medium contained 2.5 mM $Ca^{2+}$. In one series (●), 10 nM adrenaline was added between 50 min and 70 min. In the other series (○), the concentration of glucose was decreased from 15 mM to 10 mM between 50 min and 70 min. - - - -, Control experiments. Values are means ± SEM for six or seven experiments

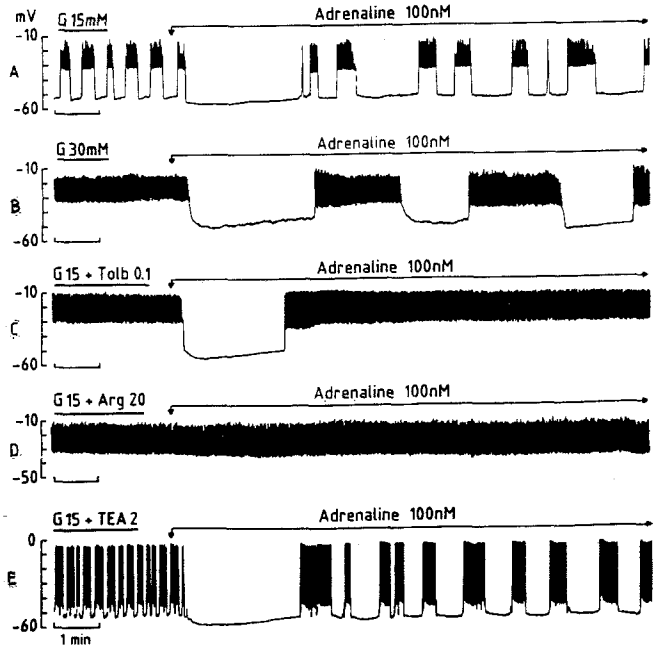


**Fig. 2A–E.** Effects of adrenaline on the electrical activity of mouse B cells. Adrenaline was added as indicated by the *arrows*. The perifusion medium contained (**A**) 15 mM glucose (*G*), (**B**) 30 mM glucose, (**C**) 15 mM glucose and 0.1 mM tolbutamide (*Tolb*), (**D**) 15 mM glucose and 20 mM arginine (*Arg*), (**E**) 15 mM glucose and 2 mM tetraethylammonium (*TEA*). The five records were obtained in different cells and are representative of five to eight experiments under each set of conditions

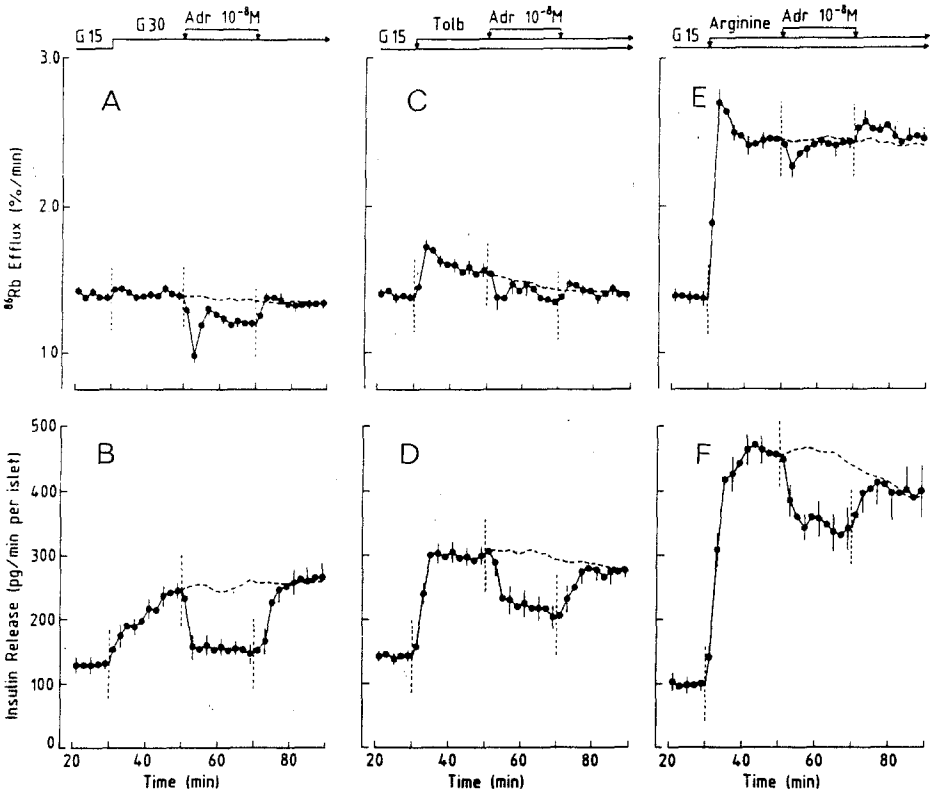


**Fig. 3A–F.** Effects of adrenaline on $^{86}Rb$ efflux and insulin release from perifused mouse islets. All experiments started in a medium containing 15 mM glucose (*G*) and adrenaline (*Adr*) was always added between 50 min and 70 min. **A–B** The concentration of glucose was raised to 30 mM at 30 min. **C–D** Tolbutamide (*Tolb*, 0.1 mM) was added to the medium at 30 min. **E–F** Arginine (20 mM) was added to the medium at 30 min. - - - -, Control experiments without addition of adrenaline. Values are means ± SEM for five or six experiments

The activity that resumed thereafter was characterized by long intervals and by slow waves that were usually of longer duration than under control conditions. In the steady state, the fraction of plateau phase (percentage of time with spike activity) was decreased from 51.9 ± 5.1% to 37.0 ± 2.2% ($n = 8$; $P < 0.005$).

Decreasing the concentration of glucose from 15 mM to 10 mM inhibited $^{86}Rb$ efflux and insulin release in a way similar to the action of adrenaline (Fig. 1). The decrease in glucose concentration was also accompanied by a transient hyperpolarization of the B cell membrane with suppression of electrical activity before reappearance of shorter slow waves and longer intervals than in 15 mM glucose (not shown) [5, 17].

*Effects of adrenaline in the presence of 30 mM glucose*

When islets were stimulated by 30 mM glucose, the B cell membrane was persistently depolarized and exhibited continuous spike activity (Fig. 2B). Adrenaline still rapidly repolarized the membrane and caused the reappearance of slow waves of very long duration. The fraction of plateau phase in the presence of adrenaline averaged 65.9 ± 4.8% ($n = 6$). Raising the concentration of glucose from 15 mM to 30 mM marginally increased $^{86}Rb$ efflux (Fig. 3A) and progressively doubled the rate of insulin release (Fig. 3B). Adrenaline still provoked a biphasic inhibition of $^{86}Rb$ efflux and inhibited insulin release. The inhibition of $^{86}Rb$ efflux was not significantly smaller than that produced in 15 mM glucose, but the percentage inhibition of insulin release was slightly reduced (42 ± 2% vs 56 ± 5%; $P < 0.05$).

*Effects of adrenaline in the presence of tolbutamide*

Tolbutamide is considered to be a specific blocker of ATP-sensitive $K^+$ channels in B cells [9, 36]. When the medium containing 15 mM glucose was supplemented with 0.1 mM tolbutamide, the B cell membrane was persistently depolarized and exhibited continuous spike activity (Fig. 2C). Adrenaline (100 nM) still transiently repolarized the membrane and abolished electrical activity in all cells. The activity that resumed thereafter, however, was again continuous in five cells, and displayed a slow wave pattern in only one cell. In the steady state, the average fraction of plateau phase (87.8 ± 7.8%) was significantly higher than that measured in 30 mM glucose and 100 nM adrenaline (65.9 ± 4.8%; $P < 0.05$). When used at the higher concentration of 1 μM, adrenaline restored slow waves in all cells (3/3) stimulated with glucose and tolbutamide (not shown). Addition of tolbutamide to the medium containing 15 mM glucose rapidly accelerated $^{86}Rb$ efflux and increased insulin release (Fig. 3C, D). The inhibition of $^{86}Rb$ efflux by adrenaline was clearly attenuated and the percentage inhibition of insulin release was smaller than in the presence of 15 mM glucose alone (26 ± 5% vs 56 ± 5%; $P < 0.01$).

*Effects of adrenaline in the presence of arginine*

Arginine depolarizes the B cell membrane because of its transport in a positively charged form [4, 18]. A persistent depolarization with continuous spike activity was observed in B cells perifused with a medium containing

15 mM glucose and 20 mM arginine (Fig. 2D). Adrenaline did not abolish electrical activity under these conditions. During the first minute, the plateau potential polarized by only 2.8 ± 0.5 mV and the frequency of the spikes decreased by 28 ± 2% ($P < 0.02$; not visible on the time scale used in Fig. 2D). Addition of arginine to the medium containing 15 mM glucose rapidly and markedly increased $^{86}Rb$ efflux and insulin release. Adrenaline only marginally decreased $^{86}Rb$ efflux in the presence of arginine and inhibited insulin release by only 24 ± 5%.

*Effects of adrenaline in the presence of TEA*

Low concentrations of TEA selectively block voltage- and $Ca^{2+}$-dependent $K^+$ channels in B cells [3, 13]. When the medium containing 15 mM glucose was supplemented with 2 mM TEA, the membrane potential still oscillated in slow waves, but the amplitude of the spikes was markedly increased (Fig. 2E). As under control conditions, adrenaline caused an initial hyperpolarization with suppression of electrical activity and, in the steady state, a marked increase in the duration of the slow waves. TEA (2 mM) did not affect the inhibition of $^{86}Rb$ efflux or insulin release caused by adrenaline in perifused islets (not shown). These results support the conclusion that voltage- and $Ca^{2+}$-dependent $K^+$ channels are not involved in the effects of adrenaline in B cells.

*Effects of adrenaline in incubated islets*

The inhibitory potency of adrenaline on insulin release was studied in greater detail with islets incubated under various conditions (Fig. 4 and Table 1). In the presence of 15 mM glucose alone, adrenaline was ineffective at 0.1 nM, inhibited release by about one-third at 1 nM and by more than 90% at 1 μM. The calculated $EC_{50}$ value was 4.6 nM. The inhibitory effects of 1–10 nM adrenaline were slightly attenuated in the presence of 30 mM glucose (Fig. 4A); the $EC_{50}$ value was almost doubled but this difference was not statistically significant (Table 1). The concentration/response relationship was much more strikingly shifted to the right when the medium containing 15 mM glucose was supplemented with 0.1 mM tolbutamide or 20 mM arginine (Fig. 4B, C). $EC_{50}$ values were increased 8- and 12-fold respectively. The potency of adrenaline was also less when insulin release was induced by 30 mM glucose plus tolbutamide or by 10 mM glucose plus arginine, than when release was stimulated by 15 mM glucose alone (Table 1). TEA (2 mM) affected neither control insulin release nor the inhibitory potency of adrenaline. Depolarization of the B cell membrane by a high concentration of extracellular $K^+$ decreased the effectiveness of adrenaline, this decrease being slightly more pronounced when ATP-sensitive $K^+$ channels had been opened by diazoxide (Table 1).

No correlation was found ($r^2 = 0.24$; $n = 62$; $P > 0.05$) between the magnitude of insulin release under control conditions and the $EC_{50}$ value for the inhibition by

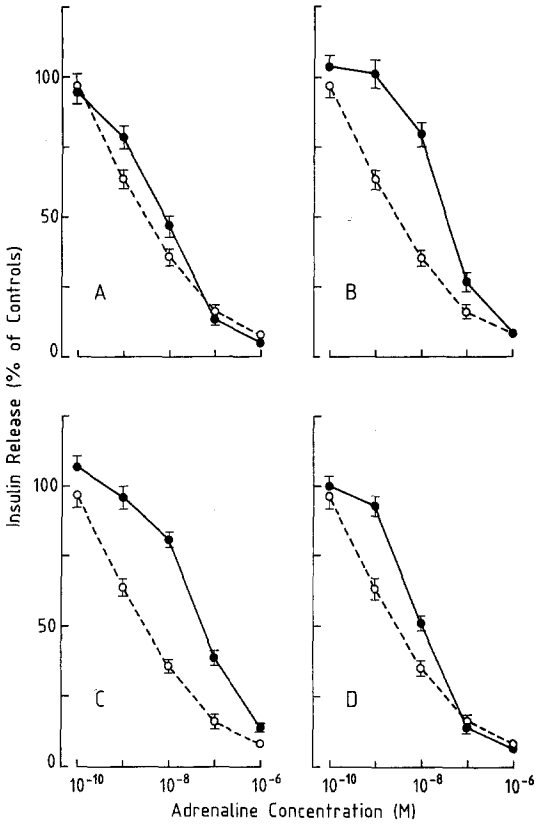


**Fig. 4A–D.** Concentration dependence of adrenaline effects on insulin release by incubated mouse islets. Batches of three islets were incubated for 60 min in 1 ml medium containing 15 mM glucose (○ in all panels) or 30 mM glucose (● in **A**), 15 mM glucose and 0.1 mM tolbutamide (● in **B**), 15 mM glucose and 20 mM arginine (● in **C**), 10 mM glucose and 5 mM arginine (● in **D**). The medium was supplemented with the indicated concentration of adrenaline. Values are means ± SEM for 20–41 batches of islets from five to eight different experiments

adrenaline. This was confirmed by the following series of experiments. First, cytochalasin B, an agent that potentiates insulin release by interacting with the microfilamentous cell web [22, 29], did not decrease the inhibitory potency of adrenaline (Table 1). Second, the combination of 10 mM glucose and 5 mM arginine did not stimulate insulin release more than 15 mM glucose, but decreased the effectiveness of adrenaline. No inhibition of insulin release was observed at 1 nM adrenaline and the inhibition by 10 nM was attenuated (Fig. 4D). The $EC_{50}$ value was increased 2.6-fold; although not significant by analysis of variance (Table 1), this difference from the control value in 15 mM glucose was highly significant ($P < 0.001$) by unpaired $t$-test.

When adrenaline was used at the high concentration of 1 μM, the inhibition of insulin release reached or exceeded 85% under all conditions (Table 1).

## Discussion

This study supports the hypothesis that a partial repolarization of the B cell membrane contributes to the inhibition of insulin release by low concentrations of

**Table 1.** Effects of adrenaline on insulin release by mouse islets incubated under various conditions[a]

| Experimental conditions | | Control insulin release (ng $h^{-1}$ $islet^{-1}$) | Inhibitory effect of adrenaline (Adr) (%) | | Inhibitory potency of adrenaline: $EC_{50}$ (nM) |
|---|---|---|---|---|---|
| Glucose (mM) | Test agent (mM) | | 1 nM Adr | 10 nM Adr | |
| 15 | – | 7.7 ± 0.4 | 36 ± 3.5 | 92 ± 0.7 | 4.6 ± 0.8 |
| 30 | – | 20.9 ± 1.1** | 23 ± 3.5* | 94 ± 0.6 | 8.9 ± 2.0 |
| 15 | Tolbutamide (0.1) | 18.1 ± 0.5** | −2 ± 4.7** | 92 ± 0.9 | 41.1 ± 8.7** |
| 30 | Tolbutamide (0.1) | 23.7 ± 0.9** | −3 ± 4.0** | 90 ± 0.9 | 36.0 ± 5.7** |
| 10 | Arginine (5) | 7.3 ± 0.3 | 8 ± 3.1** | 93 ± 0.4 | 11.9 ± 0.7 |
| 10 | Arginine (20) | 20.5 ± 0.9** | 12 ± 4.4** | 87 ± 0.8** | 22.8 ± 4.1* |
| 15 | Arginine (20) | 33.5 ± 1.5** | 3 ± 3.2** | 87 ± 0.8** | 67.0 ± 7.1** |
| 15 | TEA (2) | 8.2 ± 0.8 | 33 ± 4.4 | 88 ± 0.8* | 4.8 ± 1.9 |
| 15 | KCl (20) | 26.8 ± 1.1** | 17 ± 3.8** | 90 ± 1.1 | 24.5 ± 7.7* |
| 15 | Diazoxide (0.1) + KCl (30) | 20.1 ± 0.6** | 4 ± 3.7** | 85 ± 1.3** | 36.9 ± 7.2** |
| 15 | Cytochalasin B (0.01) | 21.1 ± 1.0** | 20 ± 3.4** | 93 ± 0.6 | 6.6 ± 0.9 |

[a] Batches of three islets were incubated for 60 min in 1 ml medium containing the indicated concentration of glucose and test substance, and supplemented with various concentrations of adrenaline (0.1 nM − 1 μM). At the end of the incubation a sample was taken for insulin assay. The concentration of adrenaline producing 50% inhibition of release ($EC_{50}$) was calculated as described in Materials and methods. Values are means ± SEM for 20 − 41 batches of islets (control release and inhibitory effect of adrenaline) or for five to eight experiments ($EC_{50}$ values). TEA, tetraethylammonium * $P < 0.05$; ** $P < 0.01$ vs effects in the presence of 15 mM glucose

adrenaline. The inhibitory potency of adrenaline was reduced by depolarizing agents that opposed the repolarizing action of the catecholamine, regardless of the mechanism by which the depolarization was produced. High concentrations of adrenaline, however, inhibited insulin release nearly completely under all conditions.

The repolarization and inhibition of electrical activity caused by adrenaline are currently ascribed to an increase in $K^+$ permeability of the B cell membrane [8], probably because of the opening of $K^+$ channels distinct from the ATP-sensitive $K^+$ channels [31]. The concomitant paradoxical decrease in $^{86}Rb$ efflux has been attributed to the fact that the expected acceleration through $K^+$ channels opened by adrenaline is opposed by the closure of voltage- and $Ca^{2+}$-activated $K^+$ channels [8]. This conclusion is supported by the observations that the effects of adrenaline on the membrane potential and on $^{86}Rb$ efflux were at least partially mimicked by a lowering of the concentration of glucose from 15 mM to 10 mM as they were by a low concentration of diazoxide [8], two changes known to open ATP-sensitive $K^+$ channels [5, 17, 36].

Both the recordings of membrane potential in single B cells and the measurements of $^{86}Rb$ efflux from whole islets show that the ability of adrenaline to repolarize the membrane and inhibit electrical activity was reduced by tolbutamide and by arginine. We previously reported that adrenaline does not affect the membrane potential or $^{86}Rb$ efflux in the presence of high $K^+$ [8]. These three experimental conditions share the property of depolarizing the B cell membrane, but do so by distinct mechanisms. A decrease in $K^+$ permeability by blockade of ATP-sensitive $K^+$ channels with tolbutamide [9, 36], the production of an inward current by the transport of positively charged arginine [4, 18], and the shift of the equilibrium potential for $K^+$ to less negative values by the increase in extracellular $K^+$ thus had similar effects. In addition, the inhibitory potency of adrenaline on insulin release was reduced under all these conditions.

The repolarization of the B cell membrane may lead to inhibition of insulin release by reducing $Ca^{2+}$ influx and eventually lowering cytosolic free $Ca^{2+}$. Such a lowering was indeed produced by agonists of $\alpha_2$-adrenoceptors in ob/ob mouse islets [1, 28] but not in insulin-secreting RINm5F cells [37]. This discrepancy is only apparent and can be explained. In the latter experiments, RINm5F cells were stimulated by alanine, which depolarizes the membrane because of its cotransport with $Na^+$ [10]. This inward depolarizing current, like that produced by arginine in the present study, was probably sufficient to prevent the small increase in $K^+$ permeability brought about by clonidine from repolarizing the membrane. Measurements of the effects of catecholamines on $^{45}Ca$ uptake also deserve critical discussion. An inhibition of $^{45}Ca$ net uptake (60 − 120 min) has been observed in several studies [15, 24, 26], but its significance was minimized because it only occurred at concentrations of the agonists higher than those required to inhibit insulin release [26]. However, these long measurements do not reflect $Ca^{2+}$ influx. Catecholamines also inhibit glucose-induced $Ca^{2+}$ influx, estimated by the 5-min uptake of $^{45}Ca$ [2, 39, 40], and this effect is already significant with 10 nM adrenaline [40].

The experimental conditions that most clearly reduced the inhibitory potency of adrenaline (which increased the $EC_{50}$ value) induced a larger control release of insulin than did 15 mM glucose (the reference condition). One could thus wonder whether it is not simply the rate of release that determines the sensitivity to adrenaline. Several arguments indicate that this is not the case. First, increasing insulin release by cytochalasin B, which acts at a late step of stimulus/secretion coupling [22, 29], did not modify the sensitivity to adrenaline. Secondly, the control rate of insulin release was similar with 15 mM

glucose or with 10 mM glucose plus 5 mM arginine, but the effectiveness of adrenaline was reduced under the latter conditions. Thirdly, several combinations (15 mM glucose plus tolbutamide, 10 mM glucose plus 20 mM arginine, 15 mM glucose plus diazoxide and high K) stimulated insulin release to a similar extent as 30 mM glucose, but markedly reduced the inhibitory potency of adrenaline. Finally, no correlation was found between the $EC_{50}$ values for adrenaline inhibition and the control rate of release.

Our observation that tolbutamide attenuated the effects of adrenaline on $^{86}Rb$ efflux and B cell membrane potential may seem to be in contradiction with the recent suggestion that the inhibitory effects of clonidine in B cells are insensitive to sulphonylureas [31]. It should, however, be noted that in the latter study glibenclamide was added to the medium less than 1 min after a high concentration of clonidine, i.e. when the effect of the $\alpha_2$-agonist on the membrane potential is the largest. The conclusion that sulphonylureas and adrenaline act on distinct $K^+$ channels [31] may be correct. On the other hand, the conclusion that sulphonylureas do not influence the inhibition of B cell function by adrenaline [31] is misleading. In the steady state, blockade of ATP-sensitive $K^+$ channels by sulphonylureas partly compensates for the increase in $K^+$ conductance brought about by activation of $\alpha_2$-adrenoceptors.

It is generally held that activation of $\alpha_2$-adrenoceptors completely inhibits insulin release regardless of the mode of action of the stimulus. However, this view is only based on in vitro experiments using high concentrations of catecholamines or of clonidine. To our knowledge, the concentration dependence of adrenaline inhibition had only been determined during glucose-stimulation of insulin release [2, 26, 30, 33, 34, 40]. The present study shows that the differential sensitivity to adrenaline inhibition is only observed at low concentrations of the catecholamine. When adrenaline was used at the high concentration of 1 μM, insulin release was inhibited by about 90%, irrespective of the conditions. Such a non-discriminative inhibition is likely to result from an action on a common pathway of stimulus/secretion coupling. The existence of this action beyond the generation of second messengers was indeed demonstrated with permeabilized islet cells [21, 35, 38].

In conclusion, we suggest that the repolarization of the B cell membrane plays a significant role in the inhibition of insulin release at least by low concentrations of adrenaline. When high concentrations are used, an effect on later steps of stimulus/secretion coupling may become predominant. Further investigations are necessary to establish whether the late effect is also produced by low concentrations of adrenaline, and whether these distinct effects are mediated by a single class of $\alpha_2$-adrenoceptors coupled to several G proteins, by receptors with variable affinities for the agonists, or by receptors of different subtypes [20]. Our in vitro findings may probably explain the observation that higher doses of adrenaline were required to inhibit tolbutamide- or arginine-induced insulin release than to inhibit glucose-induced release in man [12]. Conversely, these in vivo observations attest to the physiological relevance of the differential inhibition by low concentrations of adrenaline.

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