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SPARTEINE INCREASES INSULIN RELEASE BY DECREASING THE K^+ PERMEABILITY OF THE B-CELL MEMBRANE

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Abstract—The effects of sparteine on the pancreatic B-cell function have been studied with mouse islets. In the presence of a non-stimulatory concentration of glucose (3 mM), sparteine (0.2–1 mM) decreased the rate of $^{86}Rb^+$ efflux from islet cells, depolarized the B-cell membrane, induced a glucose-like electrical activity and stimulated insulin release. This increase in release was observed over a large range of glucose concentrations (3–20 mM), and was most marked in the presence of 10 mM glucose. At this concentration of glucose, the effect of sparteine was already detected with 0.02 mM and was maximal with 0.5 mM. Higher concentrations of sparteine only had a transient effect on insulin release. In the presence of 10 mM glucose, 0.2 mM sparteine decreased $^{86}Rb^+$ efflux and increased $^{45}Ca^{2+}$ efflux from islet cells. The effect on $^{86}Rb^+$ efflux was only transient in the presence of extracellular calcium, whereas the effect on $^{45}Ca^{2+}$ efflux required the presence of extracellular calcium. The electrical activity induced by glucose in B-cells was augmented by sparteine which, at a concentration of 0.5 mM, produced a persistent depolarization with continuous spike activity. The potentiation of insulin release by sparteine was not reversible, but was inhibited by adrenaline and completely blocked by omission of extracellular calcium. Sparteine reversed the increase in $^{86}Rb^+$ efflux and the decrease in insulin release caused by diazoxide. These results show that sparteine increases insulin release by reducing the K^+-permeability of the B-cell membrane.

A decrease in the potassium permeability of the B-cell membrane is a major step in the sequence of events leading to the stimulation of insulin release by various secretagogues, including the clinically useful sulphonylureas [1–6]. However, the nature of the K-channels involved and the mechanisms whereby their permeability is changed by insulinotropic substances remain unclear (for a review see [7]).

Several pharmacological agents, known to decrease K^+ permeability in other tissues, have been used as tools to characterize these K-channels and to clarify their role in the stimulus-secretion coupling in B cells. Quinine [8], tetraethylammonium [9] and 9-aminoacridine [10] were found to increase insulin release, and this effect could be ascribed to their ability to decrease K^+ permeability in B-cells [9–14]. On the other hand, apamine [5, 15] and aminopyridines [16] were ineffective. Within this framework, we have investigated the effects of sparteine, an alkaloid that was reported to inhibit K^+ conductance in nerve cells [17–19].

MATERIALS AND METHODS

All experiments were performed with islets of fed female NMRI mice. For electrophysiological experiments, a piece of the splenic part of the pancreas was fixed in a small perifusion chamber, and the membrane potential of single B-cells was recorded with microelectrodes [20]. B-cells were identified by the typical electrical activity that they display in the presence of 10–15 mM glucose [21]. For all other experiments, islets were isolated after collagenase digestion of the pancreas. The techniques and the dynamic system of perifusion used to measure insulin release and $^{86}Rb^+$ or $^{45}Ca^{2+}$ efflux from the islets have been described in detail [11, 22]. The perifusion medium was a bicarbonate-buffered solution [23], that was supplemented with bovine serum albumin [5 mg/ml], except for electrophysiological recordings. In certain experiments, $CaCl_2$ was omitted and was substituted for by $MgCl_2$.

Sparteine sulfate was obtained from Janssen Chimica (Beerse, Belgium) and dissolved in the appropriate solutions just before use. When necessary, the pH of the solutions was adjusted back to 7.4 with NaOH. Diazoxide (Schering Corp., Bloomfield, NJ) was first dissolved in NaOH (0.1 M) and aliquots of the stock solution were added to the appropriate media. The experiments using adrenaline (Parke–Davis, Detroit, MI) were carried out in the presence of 0.5 mM ascorbic acid.

Results are presented as means ± S.E.M. The statistical significance of differences between experimental groups was assessed by Student's *t* test for paired or unpaired data, as appropriate.

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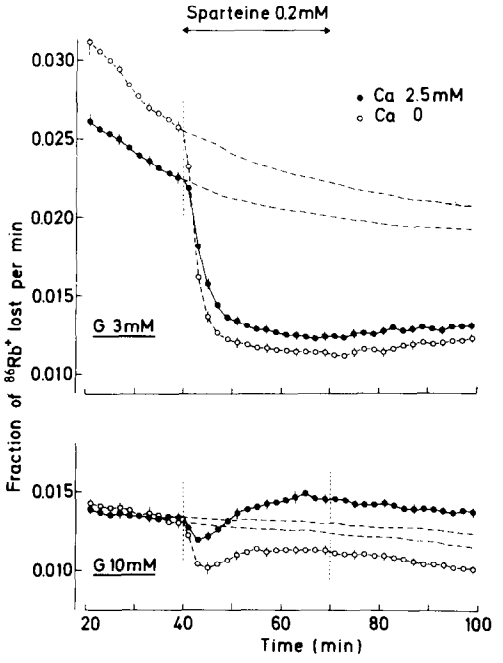

Fig. 1. Effects of sparteine on $^{86}Rb^+$ efflux from mouse islets perifused in the presence of 3 or 10 mM glucose (G). Sparteine (0.2 mM) was added, between 40 and 70 min, to a medium devoid of calcium (○) or containing 2.5 mM $CaCl_2$ (●). Control experiments without sparteine are shown by the broken lines. Values are means ± S.E.M. for four experiments.

RESULTS

Effects of sparteine in the presence of a non-stimulatory concentration of glucose

Addition of sparteine (0.2 mM) to a medium containing 3 mM glucose markedly decreased the rate of $^{86}Rb^+$ efflux from preloaded islets, but withdrawal of the drug was only followed by a very slight increase in $^{86}Rb^+$ efflux (Fig. 1, upper panel). The inhibitory effect of sparteine was more marked in the absence than in the presence of extracellular Ca^{2+}.

When the islets were perifused with a medium containing calcium and 3 mM glucose, 0.2 mM sparteine slowly depolarized the B-cell membrane and induced electrical activity (Figs 2A and B). This activity consisted of slow waves of the membrane potential with spikes superimposed on the plateau, and was qualitatively similar to that induced by a stimulatory concentration of glucose (Fig. 2C). A higher concentration of sparteine (1 mM) caused a more rapid and persistent depolarization of the B-cell membrane, with a continuous spike activity (Fig. 1C). However, the amplitude and the frequency of the spikes progressively decreased with time. Removal of sparteine was followed by disappearance of the electrical activity, but the membrane did not completely repolarize to the same level as before addition of sparteine (not shown).

Sparteine (0.2 mM) increased basal insulin release from islets perifused with 3 mM glucose by approximately 75% (Table 1).

Effects of sparteine in the presence of stimulatory concentrations of glucose

As shown by Table 1, insulin release by control islets increased by about 1.5-, 4.5- and 18-fold when

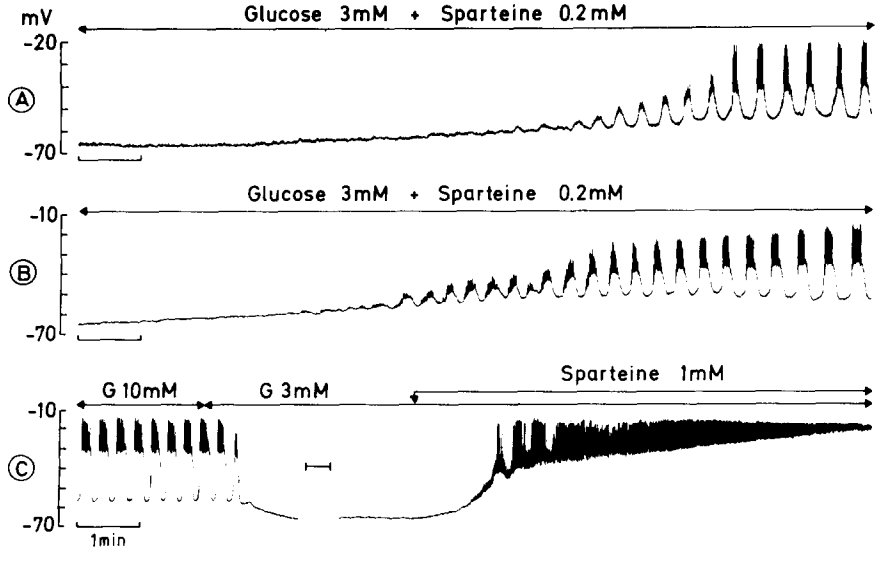

Fig. 2. Effects of sparteine on the membrane potential of single mouse B-cells perifused with a medium containing 3 mM glucose (G). Records A and B start 8 min after the glucose concentration was decreased from 10 to 3 mM and at the time of sparteine addition. The left part of record C shows the control electrical activity induced by 10 mM glucose and the hyperpolarization that follows a decrease in the glucose concentration from 10 to 3 mM. The right part is the continuation of the same record after an interruption of 4.5 min. The three records were obtained in different cells and are representative of results obtained in four to five different experiments.

Table 1. Effects of sparteine on insulin release by mouse islets perifused with various concentrations of glucose

Glucose concentration (mM)	Insulin release (ng/islet/80 min)		Stimulatory effect of sparteine (% of controls)
	Controls	Sparteine (0.2 mM)	
3	1.6 ± 0.2	2.8 ± 0.2*	176 ± 12
7	2.5 ± 0.3	11.2 ± 0.6*	455 ± 23
10	7.1 ± 0.4	33.7 ± 2.1*	475 ± 29
20	28.1 ± 1.9	48.2 ± 3.5*	172 ± 14

Sparteine was added, at the concentration of 0.2 mM, to perifusion media containing various concentrations of glucose.

The experimental protocol was similar to that shown in Fig. 3B.

Values are means ± S.E.M. for 4–6 experiments.

* $P < 0.001$ vs controls.

the concentration of glucose in the perifusion medium was raised to 7, 10 and 20 mM, respectively. Sparteine (0.2 mM) markedly potentiated the effect of the three concentrations of glucose, but the largest relative and absolute increase was observed in the presence of 10 mM glucose.

At this latter concentration of glucose, sparteine produced a dose-dependent increase in insulin release, that was already significant with 0.02 mM, was maximal with 0.5 mM, but was less marked with 2 mM (Fig. 3A). This peculiar dose-dependency was due to the time-course of the changes in release brought about by the different concentrations of sparteine (Fig. 3B). The onset of the increase in release and its magnitude during the initial minutes were directly related to the concentration of the drug, but the effect of the highest concentrations was not sustained. The rate of release reached a maximum and then started to decline after about 10, 25 and 35 min with 2, 0.5 and 0.2 mM sparteine, respectively. By contrast, the effect of the lowest concentrations steadily increased during the whole experiments. The potentiation of insulin release by 0.2 mM sparteine was not affected by 10 μM atropine or propranolol (not shown).

The potentiating effect of 0.2 mM sparteine could not be reversed by the mere withdrawal of the drug from the perifusion medium (Fig. 4A), but was reversibly suppressed by adrenaline (Fig. 4B). In the absence of extracellular calcium, the stimulation of insulin release by 10 mM glucose was inhibited and its potentiation by sparteine totally prevented (Fig. 4C). However, readmission of calcium was followed by a prompt and marked increase of the secretion rate to values similar to those measured in control islets.

Addition of 0.2 mM sparteine to a medium containing 10 mM glucose caused an initial inhibition of $^{86}Rb^{+}$ efflux from islet cells and a secondary acceleration above control values (Fig. 1, lower panel). In a Ca-free medium, the inhibitory effect of sparteine was much more pronounced and persisted during the whole experiment. Under similar conditions, sparteine strongly stimulated $^{45}Ca^{2+}$ efflux

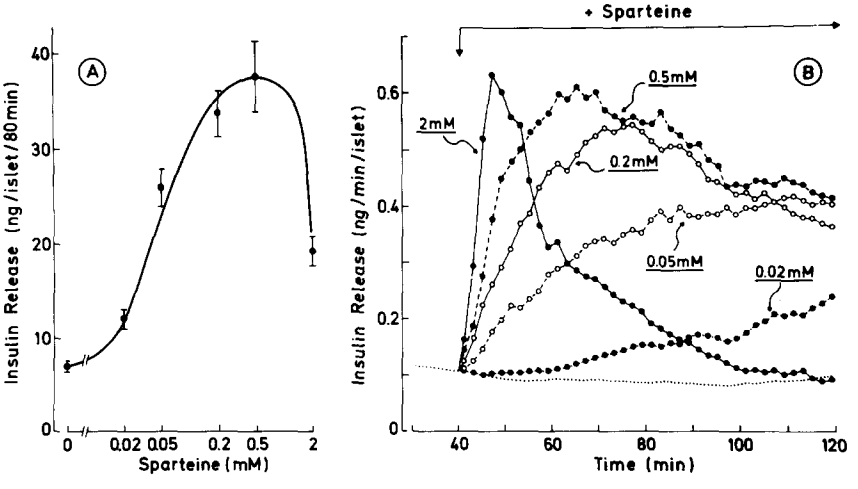

Fig. 3. Effects of various concentrations of sparteine on insulin release by mouse islets perifused with 10 mM glucose. Sparteine was added, at the indicated concentrations, between 40 and 120 min. The time course of the effects is shown by panel B, where the stippled line illustrates control experiments without sparteine. The total amount of insulin released between 40 and 120 min is shown by panel A. Values are means for four to five experiments. For the sake of clarity, S.E.M. are shown only in panel A.

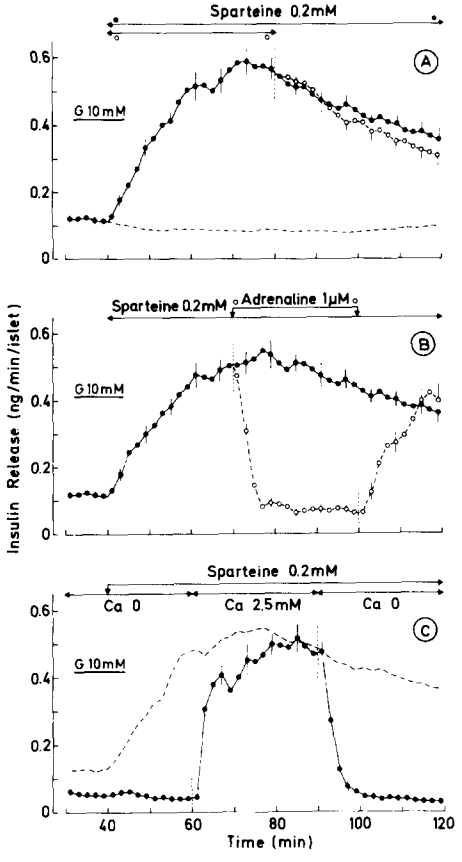

Fig. 4. Characteristics of the effects of sparteine on insulin release by mouse islets perifused with 10 mM glucose. A. Sparteine was added at 40 min and remained present either until 120 min (●) or only until 80 min (○). The broken line shows control experiments without sparteine. B. In one series of experiments (○), 1 μM adrenaline was added (between 70 and 100 min) to the medium containing sparteine. C. The experiments started in the absence of $CaCl_2$, which was added only between 60 and 90 min. Sparteine was present between 40 and 120 min. The broken line shows experiments carried out in the presence of $CaCl_2$ throughout. Values are means ± S.E.M. for four to five experiments.

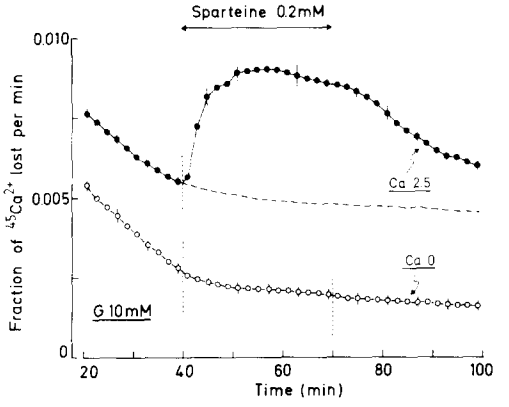

Fig. 5. Effects of sparteine on $^{45}Ca^{2+}$ efflux from mouse islets perifused in the presence of 10 mM glucose (G). Sparteine (0.2 mM) was added between 40 and 70 min to a medium devoid of calcium (○) or containing 2.5 mM $CaCl_2$ (●). Control experiments without sparteine are shown by the broken line. Values are means ± S.E.M. for four experiments.

from islets perifused in the presence of calcium, but had no effect when the medium was devoid of calcium (Fig. 5).

The electrical activity induced by 10 mM glucose in B-cells was rapidly modified by sparteine. At the concentration of 0.1 mM, the drug induced a progressive lengthening of the slow waves with spike activity and a shortening of the polarized silent intervals (Fig. 6A and B). After 8–10 min, the duration of the slow waves was approximately doubled, whereas that of the intervals was decreased by about 35% (Table 2). This did not change the frequency of the slow waves, but markedly increased the fraction of plateau phase (i.e. the percentage of time with spike activity). At the higher concentration of 0.5 mM, sparteine consistently (eight cells) caused a persistent depolarization of the B-cell membrane with continuous spike activity after a delay of 1.6 to 9.8 min (Figs 6B and C). An increase in the concentration of extracellular calcium was able, however, to restore slow waves (Fig. 6C).

Table 2. Effects of sparteine on the slow waves of membrane potential triggered by 10 mM glucose in pancreatic B-cells

	Controls	+Sparteine (0.1 mM)
Duration of slow waves (sec)	7.36 ± 0.89	15.23 ± 2.51*
Duration of intervals (sec)	15.41 ± 2.02	10.19 ± 1.34**
Frequency of slow waves (per min)	2.76 ± 0.27	2.49 ± 0.25
Fraction of plateau phase (%)	32.9 ± 3.4	58.8 ± 4.7***

The effects of sparteine (0.1 mM) were studied in six separate experiments. In each experiment, all slow waves and intervals were measured during four min, just before addition of sparteine. They were also measured between 8 and 10 min after addition of the drug. Values are means ± S.E.M. for 6 cells.

* $P < 0.02$, ** $P < 0.01$, *** $P < 0.005$ (by paired t test).

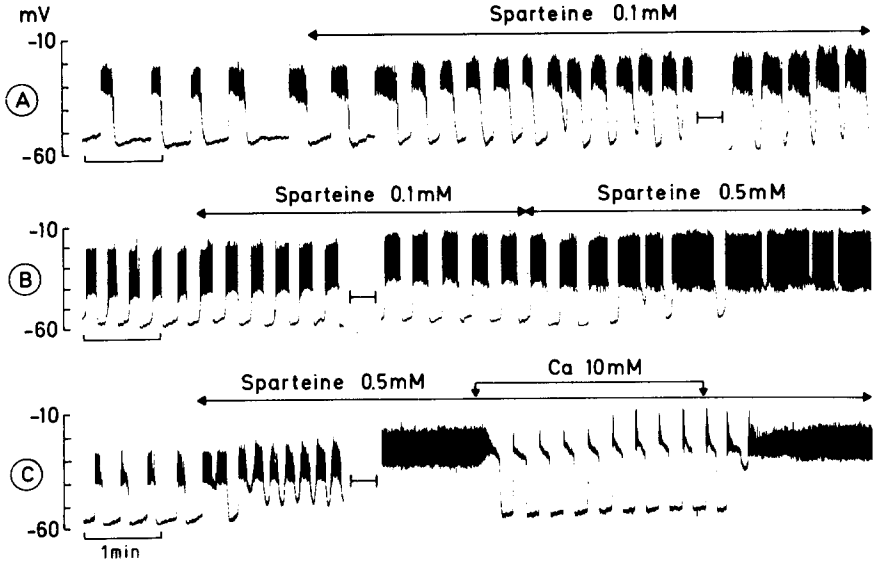

Fig. 6. Effects of sparteine on the electrical activity induced by 10 mM glucose in single mouse B-cells. Sparteine was added as indicated by the arrows and, in C, the concentration of $CaCl_2$ was raised to 10 mM for 3 min. The interruptions of the records correspond to intervals of 4, 8.5 and 7 min, in A, B and C, respectively. The three records were obtained in different cells and are representative of six to eight experiments.

Antagonistic effects of sparteine and diazoxide

In islets perifused with 15 mM glucose, 0.1 mM diazoxide caused a simultaneous increase in $^{86}Rb^+$ efflux and decrease in insulin release. Addition of sparteine to a medium containing diazoxide reversed these changes in a dose-dependent manner (Fig. 7).

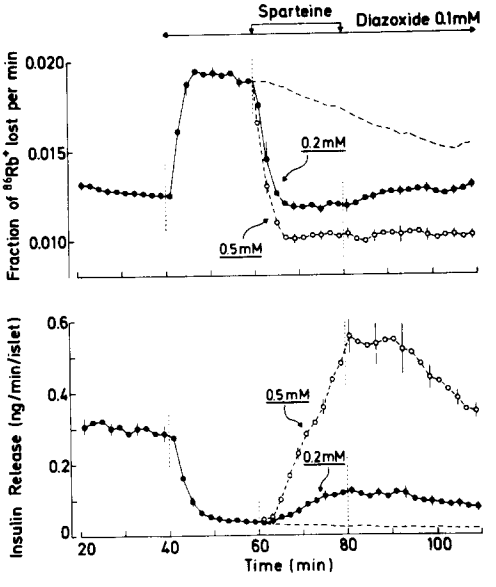

Fig. 7. Reversal by sparteine of the effects of diazoxide on $^{86}Rb^+$ efflux and insulin release from mouse islets perifused with a medium containing 15 mM glucose. Diazoxide (0.1 mM) was added between 40 and 110 min. Sparteine was added, at the indicated concentrations, only between 60 and 80 min. Values are means ± S.E.M. for four experiments.

DISCUSSION

Sparteine has been used in the management of various cardiac arythmias [24] and as an oxytocic agent [25]. The present study shows that, *in vitro* at least, it powerfully increases insulin release. It thus shares the property of another alkaloid, quinine (or quinidine), to affect markedly the function of the heart, uterus and pancreatic B-cells. We are not aware of cases of hypoglycaemia caused by sparteine. This may be due to the relatively low concentrations of the drug in the blood of treated patients (below 5 μM) and its important binding (50%) to plasma proteins [26]. It has been reported, however, that, in rat, sparteine inhibits the hyperglycaemic effect of asphyxia and of an anaesthesia with urethane, but the mechanisms were not investigated [27, 28].

In the presence of 10 mM glucose, 0.2 mM sparteine increased insulin release almost 5-fold. Though this large effect was not reversible, several lines of evidence indicate that it does not result from a damage of B-cells. First, the amount of insulin that was released during stimulation with sparteine did not exceed 12–13% of the total insulin content of the islets used. Second, the potentiating effect of sparteine could be blocked by adrenaline and totally prevented by omission of extracellular calcium. Third, sparteine caused no electrolytic or electrical signs of cellular damage. It has also been reported that the effects of sparteine on nerve [18] or heart muscle cells [29] are only slowly and partially reversible after washout of the drug. The exact site of action of sparteine is not known, but indirect evidence suggests that it may have to enter the cells and then act on the internal face of the plasma membrane [17]. This would be compatible with our observations that the onset of the effect and the time necessary to

produce a similar maximal effect are clearly dose-dependent. It is also possible that, in the presence of the highest concentration of sparteine (2 mM), an excessive accumulation of the drug causes unspecific alterations of the cell function which bring about the time-dependent decrease in the rate of insulin release.

The membrane potential of nonstimulated B-cells is mainly determined by the K^+ permeability of the plasma membrane [30]. Qualitative changes of this latter can be established by measuring $^{86}Rb^+$ efflux from islet cells in parallel with the membrane potential [7]. The combined observations that sparteine decreased $^{86}Rb^+$ efflux and depolarized the B-cell membrane strongly suggest that the drug decreases the K^+ permeability of the B-cell membrane. This decrease in K^+permeability may be considered as the cause of the depolarization, which, in turn, activates voltage-dependent Ca-channels, as shown by the appearance of an electrical activity known to be underlain by inward Ca currents [20, 31–33].

It is currently admitted that the repolarization phase of the slow waves of membrane potential induced by glucose in B-cells is, at least partially, due to an increase in K^+permeability (for review, see [7]). The lengthening of these slow waves and the decrease in duration and amplitude of the polarized intervals suggest that sparteine also reduces K^+permeability of the B-cell membrane in the presence of a stimulatory concentration of glucose. Such a conclusion is in complete agreement with the sustained decrease in $^{86}Rb^+$ efflux produced by sparteine in the absence of calcium, but apparently less compatible with the observation that sparteine only produced a transient inhibition followed by a secondary acceleration of $^{86}Rb^+$ efflux in the presence of calcium. This is, however, not an unusual situation. Thus, addition of leucine or tolbutamide to a medium containing a stimulatory concentration of glucose or simply raising the concentration of glucose accelerates $^{86}Rb^+$ efflux from the islets [1, 3, 34, 35], although the genuine effect of these substances is to decrease the K^+ permeability of the B-cell membrane. This paradoxical increase in $^{86}Rb^+$ efflux does not occur in Ca^{2+}-free solutions (a decrease may even be observed), and can be explained by the activation of K-channels by the incoming Ca^{2+}. That Ca^{2+} influx is indeed augmented by sparteine is shown by the marked increase in electrical activity and the strong Ca-dependent stimulation of $^{45}Ca^{2+}$ efflux (for review, see [36]).

We previously demonstrated [3] that the inhibition of insulin release by diazoxide is due to the ability of this sulphonamide to hyperpolarize the B-cell membrane by increasing its permeability to K^+. The evidence presented here that sparteine reverses the effects of diazoxide on $^{86}Rb^+$ efflux and insulin release adds further support to the contention that the effects of the alkaloid on the B-cell function are well due to a decrease in K^+ permeability of the plasma membrane. Incidentally, the observations that adrenaline blocks the effects of sparteine but that this latter antagonizes the effects of diazoxide confirm our previous conclusion [37] that diazoxide does not inhibit insulin release by activating α-adrenergic receptors, as first suggested [38, 39].

In conclusion, sparteine may be added to the list of pharmacological substances that increase insulin release by reducing the K^+ permeability of the B-cell membrane. This decrease causes depolarization and subsequent activation of voltage-dependent Ca-channels when the prevailing concentration of glucose is low. It permits to increase the periods of electrical activity during which Ca^{2+} enters the cell in the presence of a stimulatory concentration of glucose. One may hope that a 'structure–activity' study of putative inhibitors of K-channels will help to identify the type(s) of channels involved in the stimulus–secretion coupling in B cells and to clarify their mechanisms of control.

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