Br. J. Pharmac. (1987), **90**, 583–591

Adamantane derivatives: a new class of insulin secretagogues

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1 Adamantane derivatives were found to increase insulin release *in vitro*. Mouse islets were used to study the mechanisms and molecular requirements of that hitherto unrecognised property.

2 At a non-stimulatory concentration of glucose (3 mM), 1-adamantanamine (1 mM) reversibly inhibited ^{86}Rb efflux from islet cells, depolarized the β-cell membrane, induced electrical activity, stimulated ^{45}Ca uptake and efflux, and triggered insulin release. Omission of extracellular Ca^{2+} abolished the secretory response but only partially inhibited the acceleration of ^{45}Ca efflux.

3 At a stimulatory concentration of glucose (10 mM), 1-adamantanamine reversibly increased ^{86}Rb efflux, potentiated electrical activity (lengthening of the slow waves with spikes), augmented ^{45}Ca uptake and efflux, and increased insulin release. The effects of adamantanamine were dose-dependent, with a threshold concentration of 10 μM for stimulation of release.

4 2-Adamantanamine was as potent as 1-adamantanamine. In contrast, substitution of the amino group by a carboxyl group (1-adamantanecarboxylic acid) decreased the effectiveness by about 65%, and substitution by a hydroxyl group (1-adamantanol) suppressed it.

5 It is concluded that adamantane derivatives bearing an amino group decrease K^+ permeability of the β-cell membrane and thereby cause depolarization. This activates voltage-dependent Ca channels, permits Ca^{2+} influx and eventually stimulates insulin release. They may also mobilize cellular Ca^{2+}, but this effect is not sufficient to cause release.

Introduction

Oral administration of drugs aimed at stimulating insulin release is a cornerstone in the treatment of non-insulin-dependent diabetes. In clinical practice, all compounds currently used for this purpose belong to the family of sulphonylureas. There is no doubt that they are a valuable therapy, but their potential, albeit not frequent, side-effects and toxicity (Lebovitz & Feinglos, 1983), the profound hypoglycaemia that some of them occasionally cause (Asplund *et al.*, 1983), and their secondary failures not always due to exhaustion of insulin reserves (Groop *et al.*, 1986), have stimulated the search for alternative molecules.

During the last few years, several compounds unrelated to sulphonylureas have been found to stimulate insulin release *in vivo* and *in vitro* (Kameda *et al.*, 1981; Iwai *et al.*, 1983; Hanson *et al.*, 1985; Schnur & Morville, 1986), but their mode of action was only partially or not at all elucidated. It has also been shown recently that benzoic acid derivatives corresponding to the non-sulphonylurea moiety of glibenclamide or gliquidone, two hypoglycaemic sulphonylureas of the second generation, stimulate pancreatic β-cells by the same mechanisms as the parent molecules (Garrino *et al.*, 1985; 1986).

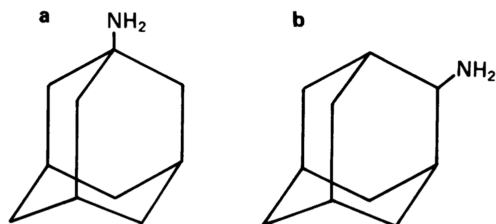

Figure 1 Structural formulae of (a) 1-adamantanamine and (b) 2-adamantanamine.

In the present study we show that simple adamantane derivatives, in particular 1- and 2-adamantanamine (Figure 1), possess the hitherto unrecognised property of stimulating insulin release. Mouse isolated

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islets were used to characterize that insulinotropic property, and to study its mechanisms by measuring the changes in ionic fluxes and β-cell membrane potential brought about by these drugs.

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 small perifusion chamber, and the membrane potential of single β-cells was continuously recorded with microelectrodes (Meissner & Schmelz, 1974). β-cells were identified by the typical electrical activity that they display in the presence of 10–15 mM glucose (Matthews *et al.*, 1973; Meissner, 1976). For all other experiments, islets were isolated after collagenase digestion of the pancreas. The techniques and the dynamic system of perifusion used to monitor the efflux of ^{45}Ca or ^{86}Rb (used as tracer for K) from preloaded islets have been described in detail (Henquin, 1979). During the experiments on ^{86}Rb efflux, a portion of each effluent fraction was withdrawn for measurement of immunoreactive insulin; rat insulin was used as a standard (Henquin & Lambert, 1975). ^{45}Ca uptake by islet cells was measured as described previously (Henquin & Lambert, 1975), using [6,6'-^{3}H]-sucrose as a marker of the extracellular space.

The medium used had the following ionic composition (mM): NaCl 120, KCl 4.8, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25. It was gassed with 94% O_2/6% CO_2 to maintain a pH of 7.4, and was supplemented with bovine serum albumin (1 mg ml^{-1}), except for electrophysiological recordings. Ca^{2+}-free solutions were prepared by replacing $CaCl_2$ by $MgCl_2$; in certain experiments 0.1 mM EGTA was also added to these Ca^{2+}-free solutions.

The following drugs were used: 1-adamantanamine hydrochloride, 2-adamantanamine hydrochloride, 1-adamantanol and 1-adamantanecarboxylic acid. They were all obtained from Janssen Chimica (Beerse, Belgium). 1-Adamantanamine and 2-adamantanamine were dissolved in the appropriate solutions just before use. Stock solutions of 1-adamantanol (50 mM in dimethylsulphoxide) and of 1-adamantanecarboxylic acid (in NaOH) were freshly prepared each day, and aliquots were added to the appropriate media. For the experiments with 1-adamantanol, the same amount of dimethylsulphoxide (1 μl ml^{-1}) was added to control solutions. ^{86}RbCl, $^{45}CaCl_2$ and [6,6'-^{3}H]-sucrose were purchased from the Radiochemical Centre (Amersham, Bucks, U.K.). All other reagents were from Merck A.G. (Darmstadt, Germany) except propranolol which was obtained from ICI (Gent, Belgium).

Electrophysiological experiments are illustrated by recordings, which are representative of the indicated number of experiments, performed with different mice. Other results are presented as means $\pm$ s.e.means for the indicated number of experiments, performed with different islet preparations. The statistical significance of differences between means of experimental groups was assessed by Student's two-tailed t test for paired or unpaired data as appropriate.

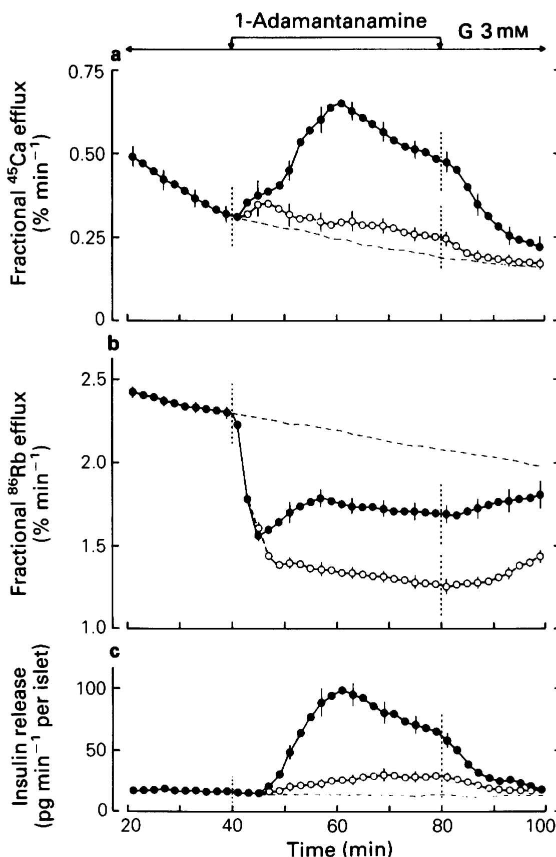

Figure 2 Effects of 1-adamantanamine on (a) ^{45}Ca efflux, (b) ^{86}Rb efflux and (c) insulin release from mouse islets perifused with a medium containing a non-stimulatory concentration of glucose (G, 3 mM). The experiments were performed in the presence of 2.5 mM Ca^{2+}. 1-Adamantanamine was added between 40 and 80 min, at a concentration of 0.5 mM (O) or 1 mM (●). Control experiments without addition of test substance are shown by the broken lines. Values are means of 5–9 experiments and vertical lines show s.e.means.

Results

Effects of 1-adamantanamine in the presence of a non-stimulatory concentration of glucose

Addition of 0.5 or 1 mM 1-adamantanamine to a medium containing 3 mM glucose and 2.5 mM Ca^{2+} inhibited ^{86}Rb efflux, accelerated ^{45}Ca efflux and stimulated insulin release from perifused islets (Figure 2). The changes in ^{45}Ca efflux and insulin release were dose-dependent, whereas the decrease in ^{86}Rb efflux was more marked with the low than with the high concentration of the drug. The changes in ^{45}Ca efflux brought about by 1 mM 1-adamantanamine clearly displayed two phases: an initial slight increase that preceded any change in release and a secondary larger acceleration, the peak of which coincided with the maximum rate of insulin release. The inhibition of ^{86}Rb efflux also followed a biphasic time-course, a late secondary rise being coincident with the stimulation of ^{45}Ca efflux and insulin release. No such late phases in ^{86}Rb or ^{45}Ca efflux occurred with 0.5 mM 1-adamantanamine. All these effects were slowly reversible upon removal of the drug (Figure 2). 1-Adamantanamine 1 mM also strongly stimulated ^{45}Ca uptake by islet cells (12.9 ± 0.7 vs 4.7 ± 0.3 pmol per islet 60 min^{-1}; $n = 12$ batches of islets; $P < 0.001$).

If the medium did not contain Ca^{2+}, 1 mM 1-adamantanamine still increased ^{45}Ca efflux and inhibited ^{86}Rb efflux, but no longer affected insulin release (Figure 3). During its initial phase the stimulation of ^{45}Ca efflux was similar to that seen in the presence of extracellular Ca^{2+}, whereas the secondary large rise was suppressed. In contrast, the inhibition of ^{86}Rb efflux was larger than in the presence of Ca^{+}. Neither the Ca-chelator EGTA (0.1 mM) nor atropine (10 μM) altered the effect of 1-adamantanamine on ^{45}Ca efflux in the Ca-free medium (not shown).

When the islets were perifused with a medium containing 3 mM glucose and 2.5 mM Ca^{2+}, 1 mM 1-adamantanamine slowly depolarized the β-cell membrane and induced electrical activity (Figure 4). This activity consisted of slow waves of the membrane potential with spikes superimposed on the plateau. It typically went through a maximum (not always characterized by a persistent depolarization to the plateau potential) and then decreased in intensity. Removal of 1-adamantanamine was followed by repolarization of the β-cell membrane (Figure 4). In one cell out of six, the drug depolarized the membrance but did not induce electrical activity (not shown).

Effects of 1-adamantanamine in the presence of a stimulatory concentration of glucose

When the perifusion medium contained 10 mM glucose and 2.5 mM Ca^{2+}, the rates of ^{45}Ca efflux and of insulin release were higher, whereas the rate of ^{86}Rb efflux was lower than in the presence of 3 mM glucose. Addition of 100 μM 1-adamantanamine reversibly increased ^{45}Ca efflux, ^{86}Rb efflux and insulin release (Figure 5). These effects were not altered by 10 μM atropine or 10 μM propranolol (not shown). The dose-dependency of the changes in ^{86}Rb efflux and insulin release brought about by 1-adamantanamine is shown

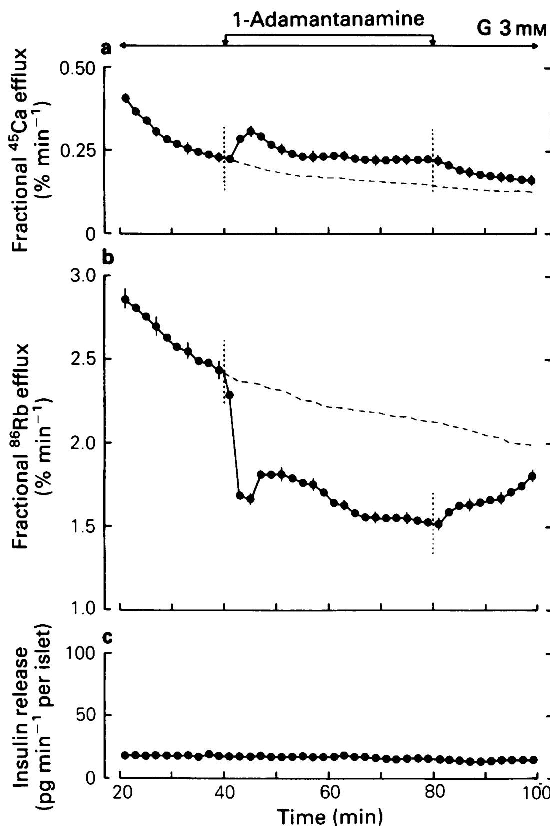

Figure 3 Effects of 1-adamantanamine on (a) ^{45}Ca efflux, (b) ^{86}Rb efflux and (c) insulin release from mouse islets perifused with a medium containing a non-stimulatory concentration of glucose (G, 3 mM). The experiments were performed in the absence of Ca^{2+}. 1-Adamantanamine (1 mM) was added between 40 and 80 min. Control experiments without addition of test substance are shown by the broken lines. Values are means of 5–7 experiments and vertical lines show s.e.means.

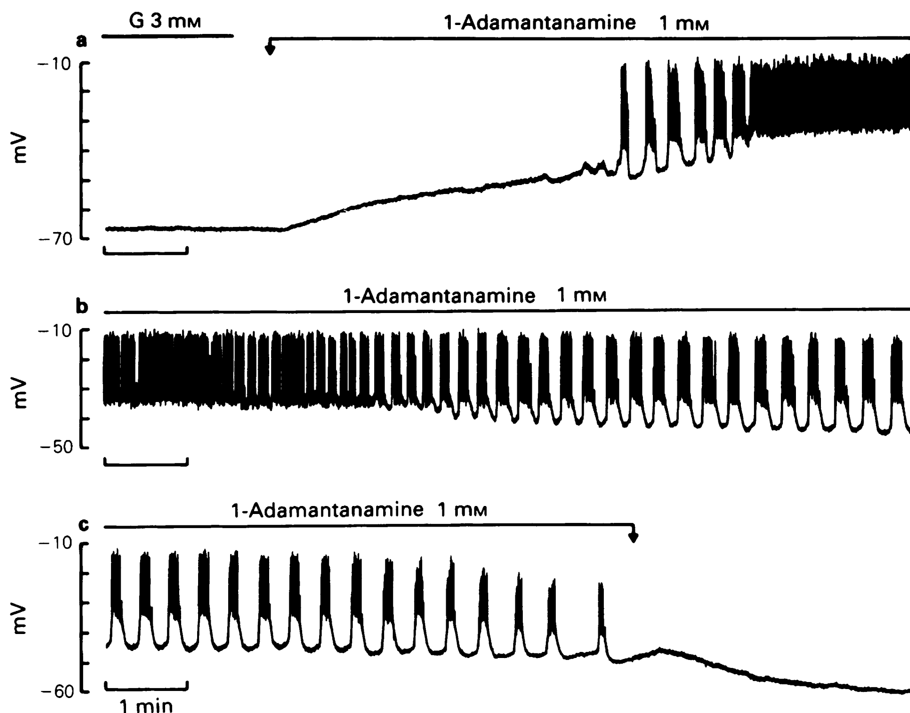

Figure 4 Effects of 1-adamantanamine on the membrane potential of a mouse β-cell perifused with a medium containing 3 mM glucose (G). 1-Adamantanamine (1 mM) was added as indicated by the arrows. The whole experiment is shown without interruption. This record is representative of 5 experiments.

in Figure 6. The lowest concentration causing a significant increase in ^{86}Rb efflux was 25 μM ($P < 0.05$), but insulin release was already significantly augmented by 10 μM 1-adamantanamine ($P < 0.02$). ^{45}Ca uptake by islet cells was increased from 13.6 ± 0.7 pmol per islet 60 min^{-1} in controls to 19.9 ± 0.7 pmol per islet 60 min^{-1} ($n = 12$ batches of islets; $P < 0.001$) in the presence of 100 μM 1-adamantanamine.

Omission of Ca^{2+} from the control medium containing 10 mM glucose barely affected ^{86}Rb efflux, but decreased ^{45}Ca efflux and prevented the stimulation of insulin release. Under these conditions, 100 μM 1-adamantanamine increased ^{45}Ca and ^{86}Rb efflux, and did not change insulin release (Figure 5). The effect on ^{45}Ca efflux was considerably smaller than in the presence of Ca^{2+}, was only short-lived, but was seen consistently. The effect on ^{86}Rb efflux was slightly smaller and less sustained than in a Ca^{2+}-medium, and persisted in the presence of 0.1 mM EGTA (not shown).

1-Adamantanamine increased the electrical activity induced by 10 mM glucose in β-cells (Figure 7). This increase was characterized by a progressive lengthening of the slow waves with spike activity and a shortening of the polarized silent intervals. In six cells, 20 min after addition of the drug the average duration of the slow waves was increased by 85 ± 15% ($P < 0.001$), whereas the average duration of the intervals was decreased by 32 ± 4% ($P < 0.005$, paired t test). This resulted in an increase in the fraction of plateau phase, i.e. the percentage of time spent at the plateau potential with spike activity (Figure 7c).

Effects of various adamantane derivatives

In the presence of 3 mM glucose and 2.5 mM Ca^{2+}, 1 mM 2-adamantanamine inhibited ^{86}Rb efflux and stimulated insulin release (Table 1). In a Ca^{2+}-free medium the inhibition of ^{86}Rb efflux was larger, but the stimulation of insulin release was abolished (not

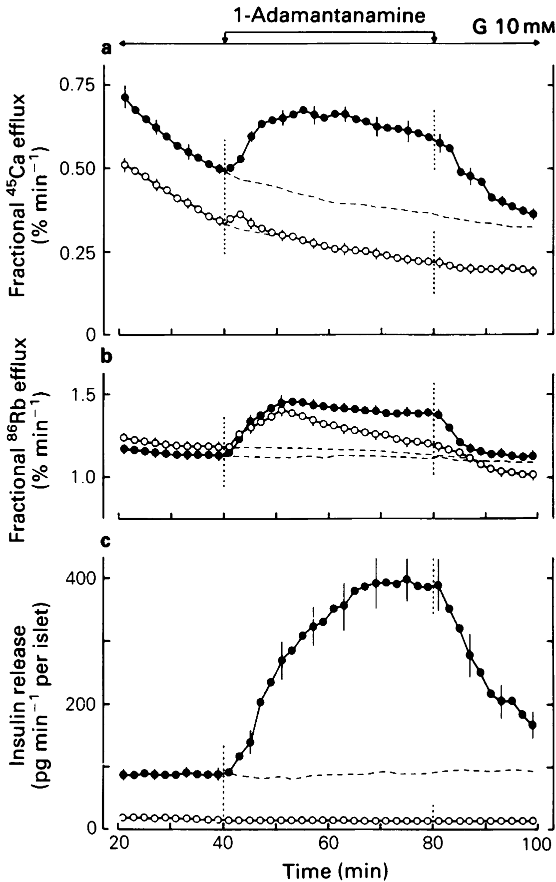

Figure 5 Effects of 1-adamantanamine on (a) ^{45}Ca efflux, (b) ^{86}Rb efflux and (c) insulin release from mouse islets perifused with a medium containing a stimulatory concentration of glucose (G, 10 mM). The experiments were performed in the presence of 2.5 mM Ca^{2+} (●), or in the absence of Ca^{2+} (○). 1-Adamantanamine (100 μM) was added between 40 and 80 min. Control experiments without addition of test substance are shown by the broken lines. Values are means of 5–7 experiments and vertical lines show s.e.means.

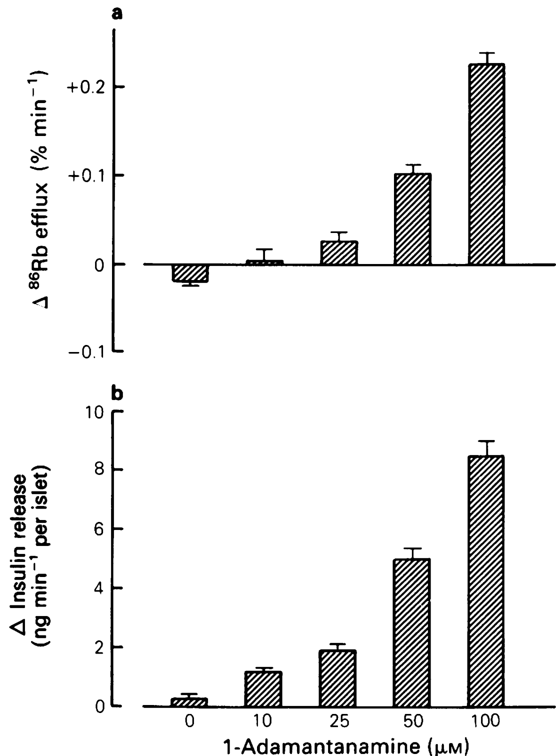

Figure 6 Effects of different concentrations of 1-adamantanamine on (a) ^{86}Rb efflux and (b) insulin release from mouse islets perifused with a medium containing 10 mM glucose and 2.5 mM Ca^{2+}. The results were obtained in experiments similar to those shown in Figure 5. The effect on ^{86}Rb efflux is given as the difference between the actual efflux rate immediately before and 20 min after addition of the drug. Total insulin release during the 40 min of stimulation was calculated after subtraction of basal release, measured immediately before addition of the drug. Values are means of 5–7 experiments and vertical lines show s.e.means.

shown). All these effects of 2-adamantanamine displayed the same time course as those of 1-adamantanamine and no statistically significant difference was found between them. In the presence of 10 mM glucose and 2.5 mM Ca^{2+}, 50 μM 2-adamantanamine increased ^{86}Rb efflux and insulin release as did 50 μM 1-adamantanamine (Table 1). As with this latter compound, a slight stimulation of release was also observed with 10 μM 2-adamantanamine (not shown).

1-Adamantanecarboxylic acid (1 mM) decreased the rate of ^{86}Rb efflux but did not stimulate insulin release at 3 mM glucose (Table 1). These effects were similar to those observed with 250 μM 1-adamantanamine. In the presence of 10 mM glucose, 50 μM 1-adamantanecarboxylic acid increased ^{86}Rb efflux and insulin, with a potency between those of 10 and 25 μM 1-adamantanamine. On the other hand, 1-adamantanol was without significant effect on ^{86}Rb efflux and insulin release under the same conditions (Table 1).

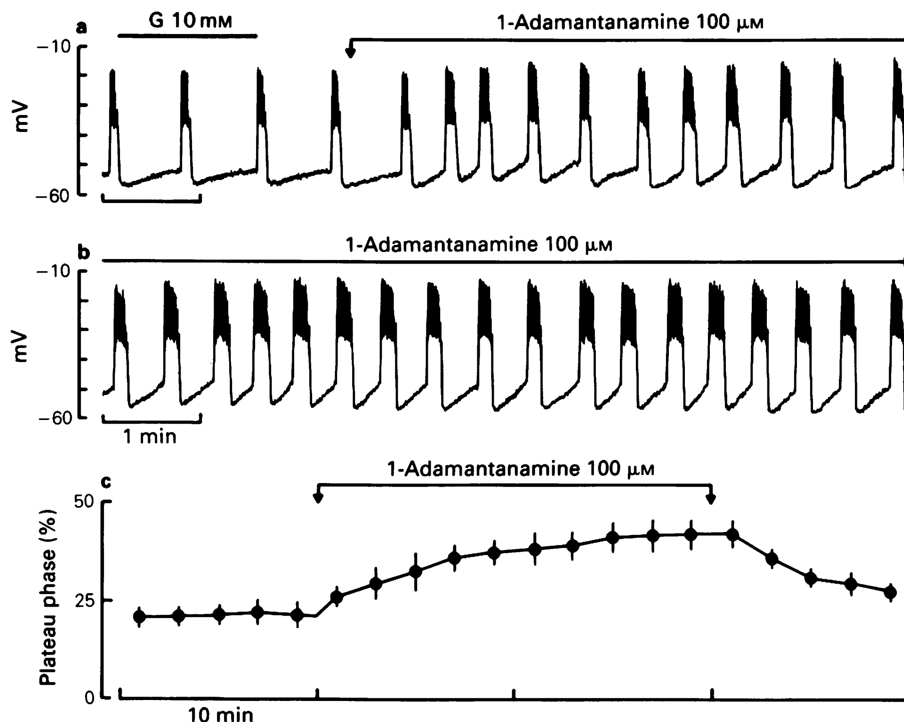

Figure 7 Effects of 1-adamantanamine on the membrane potential of a mouse β-cell perifused with a medium containing 10 mM glucose (G). 1-Adamantanamine was added as indicated by the arrows. The second record (b) is the continuation of the first one after an interruption of 7 min. These records are representative of 6 experiments. The lower part of the figure (c) shows the percentage of time spent at the plateau potential (with spike) activity) by these 6 cells. In (c) values are means and vertical lines show s.e.means.

Table 1 Effects of adamantane derivatives on ^{86}Rb efflux and insulin release from mouse islets

Test substances	n	Δ *^{86}Rb efflux* (%)	Δ *Insulin release* (ng 40 min^{-1} per islet)
Glucose 3 mM	5	−0.146 ± 0.034	−0.07 ± 0.04
+ 1-Adamantanamine (1 mM)	9	−0.566 ± 0.080**	1.89 ± 0.23***
+ 2-Adamantanamine (1 mM)	5	−0.358 ± 0.080*	2.22 ± 0.52**
+ 1-Adamantanecarboxylic acid (1 mM)	4	−0.776 ± 0.100***	−0.05 ± 0.03
Glucose 10 mM	5	−0.020 ± 0.004	0.32 ± 0.08
+ 1-Adamantanamine (50 μM)	5	0.105 ± 0.009***	5.00 ± 0.39***
+ 2-Adamantanamine (50 μM)	5	0.118 ± 0.016**	5.51 ± 0.38***
+ 1-Adamantanecarboxylic acid (50 μM)	4	0.044 ± 0.009*	1.60 ± 0.15**
+ 1-Adamantanol (50 μM)	4	−0.003 ± 0.005	0.71 ± 0.12

All experiments were similar to those shown in Figures 2 and 5, with test agents added to the medium between 40 and 80 min. For each experiment, the difference in ^{86}Rb efflux rate was calculated between 60 and 40 min (i.e. 20 min after and just before addition of test substance). Insulin release was calculated as the difference between the actual release over 40 min of stimulation and a basal release extrapolated from the rate of release measured just before addition of test substance. Values are means ± s.e.means for (*n*) experiments. *$P<0.05$, **$P<0.005$ and ***$P<0.001$ vs controls with glucose alone.

Discussion

1-Adamantanamine (Amantadine) is currently used for the clinical treatment of Parkinson's disease or drug-induced extrapyramidal disorders, and for the prophylaxis of influenza A infection (Vernier *et al.*, 1969; Parkes, 1974; Bailey & Stone, 1975). The present study shows that, *in vitro*, it increases insulin release. Hypoglycaemia is not listed among the untoward or toxicological effects of the drug (Vernier *et al.*, 1969; Parkes, 1974). However, one must bear in mind that plasma levels of 1-adamantanamine ($1-5\,\mu$M) normally remain below the threshold concentration ($10\,\mu$M) found to potentiate glucose-induced insulin release. Moreover, much higher concentrations of the drug are required to produce this effect at low glucose levels.

A number of pharmacological actions have been ascribed to 1-adamantanamine: dopaminergic effects that may be secondary to dopamine release or due to direct activation of dopamine receptors (Parkes, 1974; Bailey & Stone, 1975); indirect β-adrenergic effects (Van Ackern *et al.*, 1975; Freeman *et al.*, 1985); anticholinergic effects both at nicotinic (Warnick *et al.*, 1982) and muscarinic receptors (Freeman *et al.*, 1985). None of these mechanisms can explain the effects of 1-adamantanamine on β-cells for the following reasons. Dopamine does not stimulate, but inhibits insulin release (Quickel *et al.*, 1971). Blockade of β-adrenoceptors by propranolol did not prevent the response to 1-adamantanamine. Activation, not inhibition, of muscarinic receptors in β-cells increases insulin release (Nenquin *et al.*, 1984). Incidentally, antimuscarinic effects of 1-adamantanamine on β-cells must be very weak, if present, since $100\,\mu$M of the drug did not affect the response to $1\,\mu$M acetylcholine (unpublished observations). A weak agonist action can also be ruled out by the insensitivity of the effects of 1-adamantanamine to atropine.

The membrane potential of resting β-cells is determined primarily by the high relative K^+ permeability of the membrane (Henquin & Meissner, 1984; Matthews, 1985). Qualitative changes in membrane K^+ permeability can be established by measuring ^{86}Rb efflux from islet cells in parallel with the membrane potential. In the presence of a non-stimulatory concentration of glucose, 1-adamantanamine decreased ^{86}Rb efflux and depolarized the β-cell membrane. The combination of these effects strongly suggests that the drug decreases K^+ permeability of the β-cell membrane and, thereby, causes depolarization. When this latter effect is large enough (with 1 mM 1-adamantanamine), voltage-dependent Ca channels are activated and Ca^+ influx ensues as indicated by (a) the appearance of an electrical activity known to be due to inward Ca^{2+} currents (Henquin & Meissner, 1984; Matthews, 1985), (b) the Ca^{2+}-dependent stimulation of ^{45}Ca efflux, and (c) the increase in ^{45}Ca uptake by islet cells. Unlike the stimulation of ^{45}Ca efflux or insulin release, the inhibition of ^{86}Rb efflux by 1-adamantanamine was not directly dose-dependent, and was larger in the absence than in the presence of Ca^{2+}. A similar phenomenon has been observed with sulphonylureas and related substances (Henquin & Meissner, 1982; Matthews & Shotton, 1984a; Garrino *et al.*, 1985; 1986). It can be ascribed to activation of K channels by incoming Ca^{2+} (Henquin, 1979) and by the greater depolarization of the membrane (Matthews & Shotton, 1984b).

In contrast to sulphonylureas (Hellman, 1981; Lebrun *et al.*, 1982; Garrino *et al.*, 1986), 1-adamantanamine still increased ^{45}Ca efflux from islets perifused with a Ca-free medium. The drug thus appears to possess the additional property of mobilizing intracellular Ca^{2+}, by a mechanism that remains unknown. The observation that insulin release was not stimulated by Ca^{2+} mobilization may seem surprising but is not without precedent. A fairly similar situation occurs with acetylcholine (Nenquin *et al.*, 1984). However, this does not exclude the possibility that the effect of the drug on intracellular Ca^{2+} stores may contribute to the secretory response once Ca^{2+} influx has been stimulated.

The Ca-dependency of the electrical activity triggered by glucose in β-cells is well established (Henquin & Meissner, 1984; Matthews, 1985). The potentiation of this activity, the Ca^{2+}-dependent acceleration of ^{45}Ca efflux and the stimulation of ^{45}Ca uptake all support the contention that 1-adamantanamine also increases Ca^{2+} influx in the presence of a stimulatory concentration of glucose. It is generally accepted that an increase in K^+ permeability contributes to the repolarization phase of the slow waves of membrane potential induced by glucose (Henquin & Meissner, 1984; Matthews, 1985). The lengthening of these slow waves and the shortening of the polarized intervals again argue in favour of an inhibition of K^+ permeability by 1-adamantanamine. The paradoxical acceleration of ^{86}Rb efflux that the drug produced under these conditions was not unexpected and does not necessarily contradict the above interpretation. It is seen whenever a substance augments Ca^{2+} influx and depolarization in β-cells, whose membrane is already depolarized by a decrease in K^+ permeability (Henquin, 1980; Henquin & Meissner, 1982; Matthews & Shotton, 1984 a,b; Garrino *et al.*, 1985; 1986). Usually, however, this increase in ^{86}Rb efflux is abolished by omission of extracellular Ca^{2+} and not only attenuated as observed here with 1-adamantanamine. One tentative explanation is that Ca^{2+} mobilization, albeit small, is large enough to activate K channels.

At concentrations similar to those used in this study, 1-adamantanamine (Freeman *et al.*, 1985) and other adamantane derivatives (Meszaros *et al.*, 1981; 1982)

were found to inhibit K channels in cardiac cells, an effect that was followed by a prolongation of the action potential and resulted in a positive inotropic effect. The exact site of action of adamantane derivatives is not known. The onset of the effects of 1-adamantanamine and their ability to be reversed after withdrawal of the drug were relatively slow. This may suggest that the drug, known to permeate biological membranes (Parkes, 1974), has to enter β-cells to act at the inner side of K channels.

Structure-activity studies have shown that the effectiveness of various adamantane derivatives may vary greatly in a given system (Parkes, 1974; Warnick *et al.*, 1982). We found here that the position of the amino group was of no importance, but that a less active or a completely inactive molecule was obtained if the amino group was replaced by a carboxyl or a hydroxyl group, respectively. At 3 mM glucose, 1 mM 1-adamantanecarboxylic acid decreased ^{86}Rb efflux less markedly than did the lowest concentration (500 μM) of adamantanamine able to induce insulin release. Its inhibitory effect was paradoxically larger than that of 1 mM 1-adamantanamine because the genuine effect of this latter concentration was opposed by activation of K channels by incoming Ca^{2+} (see discussion above). Nevertheless, the interesting point is that 1-adamantanecarboxylic acid produces the same basic effect as 1-adamantanamine. This suggests that pharmacological blockade of K channels can be achieved by interaction with two distinct gating sites. However, one cannot rule out the alternative explanation that the nature of the substituent simply determines the permeability across the membrane ($NH_2 > COOH >> OH$), and that the adamantane structure itself interacts with the inner side of K channels. Experiments using the patch-clamp technique may help to resolve this question.

In conclusion, this study demonstrates that 1-adamantanamine decreases K^+ permeability of the β-cell membrane and thereby causes depolarization. This activates voltage-dependent Ca channels, permits Ca^{2+} influx and eventually stimulates insulin release. The same sequence of events is also believed to underlie the effects of sulphonylureas of the first and second generation (Henquin, 1980; Henquin & Meissner, 1982; Matthews & Shotton, 1984a; Ferrer *et al.*, 1984) and of benzoic acid derivatives of the sulphonylureas of second generation (Garrino *et al.*, 1985; 1986). In contrast to all these agents, 1-adamantanamine may also affect intracellular Ca^{2+} stores without this effect being sufficient to trigger release. It is unlikely that 1-adamantanamine itself will ever be useful in the treatment of diabetes. However, derivatives of this molecule or addition of this molecule to already active compounds may perhaps provide useful drugs. Anyhow, adamantane derivatives are, by far, the simplest molecules able to inhibit K channels in β-cells. They may thus prove useful tools to elucidate the gating mechanisms of these K channels, so important for stimulus-secretion coupling.

This work was supported by grant 5.4546.86 from the FRSM, Brussels, and by the Deutsche Forschungsgemeinschaft, SFB 246. J.C.H. is Maître de Recherches of the FNRS, Brussels. We are grateful to Prof. H. Meves for support, to Mr Gérard and W. Schmeer for skilled assistance and to S. Meerkens for editorial help.

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(*Received September 13, 1986.
Revised November 4, 1986.
Accepted November 7, 1986.*)