J. Pharm. Pharmacol. 1986, 38: 778–780
Communicated February 13, 1986

Effects of sulphonylureas on spontaneous motility and induced contractions in rat isolated uterus

A. VILLAR*, M. D. IVORA, M. P. D'OCON, E. ANSELMI, *Departmento de Farmacognosia y Farmacodinamia, Facultad de Farmacia, Avda. Blasco Ibáñez, 13, 46010 Valencia, Spain*

To clarify the action of sulphonylureas on calcium, the effect of tolbutamide and glibenclamide has been investigated on a Ca-dependent process, the contractile activity of uterine smooth muscle. Both sulphonylureas antagonized the contractions evoked by $CaCl_2$ in a non-competitive manner when the uterus was maintained in depolarizing solution and did not affect the spontaneous contractions of rat uterus. The capacity of tolbutamide and glibenclamide to relax vanadate-induced contraction of rat uterus in Ca-free medium suggests that sulphonylureas may have an intracellular site of action related to cytosolic free Ca levels, or effect a reduction in Ca action.

Although the sulphonylureas have long been in clinical use as hypoglycaemic agents, their mode of action has not yet been resolved. Like glucose, the hypoglycaemic sulphonylureas induce insulin release by raising the concentration of cytoplasmic Ca^{2+} in the pancreatic β-cells (Hellman 1982; Matthews & Shotton 1984).

The mechanism by which sulphonylureas facilitate Ca^{2+} influx in β-cells is not clear. It has been proposed that the hypoglycaemic sulphonylureas promote the entry of Ca^{2+} by acting as Ca^{2+} ionophores (Couturier & Malaisse 1980; Deleers et al 1983). Accumulating evidence suggests that the stimulation of insulin release results from sulphonylurea binding to the surface of the β-cells, which induces alteration of the ion permeability of the plasma membrane (Hellman 1982). It has been demonstrated that the hypoglycaemic sulphonylureas induce the depolarization of the β-cells which, by opening potential-dependent channels, stimulate the entry of Ca^{2+} (Henquin & Meissner 1982; Matthews & Shotton 1984).

In an attempt to clarify the action of sulphonylureas on calcium, we have studied their effect on a Ca-dependent process, the contractile activity of smooth muscle, and to do so we have investigated the action of tolbutamide and glibenclamide on Ca-induced contractions of the K^+-depolarized uterus of the rat, their action on vanadate-induced contraction in Ca-free medium and, finally, the effect of both sulphonylureas on spontaneous contractions of the rat uterus.

Materials and methods

Uterine strips were obtained from adult, female Wistar rats 150–220 g, that were treated with oestradiol benzoate (5 mg kg^{-1}) 24 h before the experiments and killed by a blow on the head. Uterine strips, freed from adhering tissue, were suspended in 30 ml organ baths containing physiological Ringer solution maintained at 31 °C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2.

To assess the effect of sulphonylureas on the influx of Ca^{2+} through voltage-sensitive channels, the strips were bathed for 20 min in Jalon Ringer solution (composition, mM: NaCl 154, KCl 5·63, $CaCl_2$ 0·648, $NaHCO_3$ 5·95 and glucose 2·77), with a resting tension of 1 g, and then exposed for 1 h to high-K^+, Ca^{2+}-free depolarizing solution (composition, mM: KCl 100, $MgCl_2$ 2·10, $NaHCO_3$ 5·95 and glucose 2·77). Cumulative amounts of $CaCl_2$ (0·025 to 25·6 mM) were introduced into the bath (Van Rossum 1963). Two cumulative dose-response curves to $CaCl_2$ were made, followed by a third dose-response curve in the presence of different concentrations of tolbutamide (4×10^{-3}, 4×10^{-4} and 4×10^{-5} M) or glibenclamide (8×10^{-5}, 4×10^{-5} and 10^{-5} M). The drugs tested were left in contact with the tissue for 15 min. Each preparation was exposed to only one concentration of the sulphonylurea.

We also studied the action of sulphonylureas on vanadate-induced contraction of rat uterus in Ca-free medium, using the method of Sakai et al (1981). The uterus was immersed for 1 h in Locke Ringer solution (composition, mM: NaCl 154, KCl 5·63, $CaCl_2$ 2·16, $MgCl_2$ 2·10, $NaHCO_3$ 5·95 and glucose 5·55) and equilibrated with a resting tension of 0·5 g. Then it was exposed for 50 min to Ca-free solution with 3 mM EDTA to deplete the tissue of calcium and, finally, this solution was replaced by Ca-free solution containing 1 mM EDTA (composition was the same as Locke Ringer solution except that $CaCl_2$ was omitted and was supplemented with 3 mM or 1 mM EDTA). After 20 to 30 min, a Ca-free contraction was induced by addition of 8×10^{-5} M vanadate. When this had reached its plateau (about 10 min), cumulative dose-response curves to tolbutamide ($3{\cdot}12 \times 10^{-5}$ to 5×10^{-4} M) or glibenclamide (5×10^{-6} to 8×10^{-5} M) were made, obtaining dose-related relaxations.

Another set of experiments was carried out to evaluate the action of sulphonylureas on spontaneous contractions. The uterine horn was immersed in Krebs-Henseleit solution (composition, mM: NaCl 154, KCl 5·63, $CaCl_2$ 2·16, $MgSO_4 \times 7\ H_2O$ 0·02, $NaHCO_3$ 5·95 and glucose 2·75), with a resting tension of 1 g, until stabilization of spontaneous contractions was reached, then the sulphonylurea was added cumulatively to the

organ bath. The doses used were: tolbutamide 2·5 × 10^{-4} to 4 × 10^{-3} M and glibenclamide 5 × 10^{-6} to 8 × 10^{-5} M.

Statistical analysis. Contractions were expressed as a percentage of the theoretical maximal effect (Emax) and relaxations were expressed as a percentage of the maximum tension obtained by vanadate addition. Emax and EC50 were calcualted using the mathematical method of Basulto et al (1978).

Statistical significance of differences between the means was assessed using Student's *t*-test for unpaired or paired data. *P* values of less than 0·05 were considered to represent significant differences. The results are presented as the mean ± s.e.

Drugs used. Tolbutamide and glibenclamide were a generous gift from Hoeschts Ibérica. Vanadate was purchased from Sigma Chemical Company. All other reagents were of analytical grade.

Apparatus. Mechanical responses of the myometrium were recorded isometrically on a recorder (680M HP), an amplifier (8805C HP) and a force dispalcement transducer (Gould Statam UC2).

Results

Effect of pre-incubation with sulphonylureas on cumulative dose-response curves to $CaCl_2$ in a depolarizing Ca-free medium. Cumulative concentration-response curves to $CaCl_2$ (0·025 to 25·6 mM) in rat uterus immersed in K^+-depolarizing Ca^{2+}-free solution were reproducible at 60 min intervals.

The uterine contractile response to calcium were inhibited by pre-incubation with sulphonylureas. Doses of tolbutamide (4 × 10^{-3} and 4 × 10^{-4} M) and glibenclamide (8 × 10^{-5} and 4 × 10^{-5} M) produced a significant reduction of Emax of dose-response curves for $CaCl_2$ without significantly affecting the EC50. The smaller doses of tolbutamide (4 × 10^{-5} M) and glibenclamide (10^{-5} M) that were used did not modify significantly the parameters of dose-response curves to $CaCl_2$ (Table 1).

Relaxant effect of sulphonylureas on vanadate-induced contraction in Ca-free medium. In Ca-free, EDTA-containing solution, the vanadate (8 × 10^{-5} M) produced a maintained contraction that was abolished by sulphonylureas as shown in Fig. 2. The addition of cumulative doses of tolbutamide (3·12 × 10^{-5} to 5 × 10^{-4} M) or glibenclamide (5 × 10^{-6} to 8 × $1(^{-5}$ M) induced dose-dependent relaxations.

The dose-response curves were made and the EC50 values were: (1·07 ± 0·09) × 10^{-4} M for tolbutamide and (1·99 ± 0·13) × 10^{-5} M for glibenclamide.

Effect of sulphonylureas on spontaneous contractions. In oestrogen-dominated rat uterus incubated in Krebs-Henseleit solution, rhythmic contractile responses were obtained. These spontaneous contractions had a stable intensity and duration.

As shown in Fig. 1 the addition of cumulative doses of tolbutamide (0·25 to 8) × 10^{-3} M or glibenclamide (0·5 to 8) × 10^{-5} M to the organ bath did not modify the

Table 1. Parameters of dose-response curves to $CaCl_2$ in absence (control) and presence of different concentrations of tolbutamide or glibenclamide.

	Drug (M)	Emax	EC50 (×10^{-4} M)
Tolbutamide	Control	100	46·3 ± 7·5
	4 × 10^{-3}	29·7 ± 13·7*	122·9 ± 52·1
	4 × 10^{-4}	64·9 ± 3·2*	34·4 ± 13·8
	4 × 10^{-5}	77·7 ± 9·8	24·4 ± 5·3
Glibenclamide	Control	100	43·2 ± 5·0
	8 × 10^{-5}	29·8 ± 7·4*	57·3 ± 30·8
	4 × 10^{-5}	53·3 ± 11·1*	26·6 ± 8·4
	1 × 10^{-5}	75·0 ± 12·8	27·5 ± 4·3

Means ± s.e. (number of experiments = 6). * $P < 0.05$ with Student's *t*-test for paired (Emax) or unpaired (EC50) data.

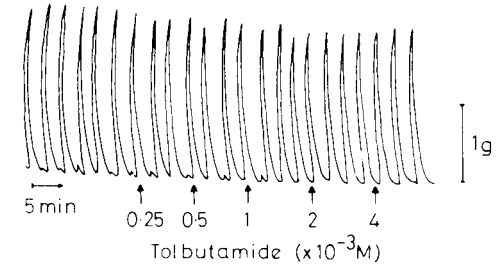

FIG. 1. Effect of tolbutamide on spontaneous contractions of rat uterus incubated in Krebs-Henseleit solution. Figure refers to final concentration of tolbutamide (M) in the bath. Number of experiments = 6.

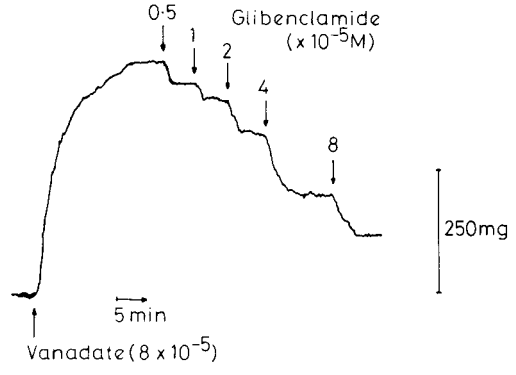

FIG. 2. Relaxant effect of glibenclamide in vanadate-induced contraction in Ca-free medium. Figure refers to final concentration of vanadate and glibenclamide (M) in the bath. Number of experiments = 6.

spontaneous contractions of rat uteri immersed in Krebs-Henseleit solution.

Discussion

Sulphonylureas may increase insulin secretion by elevating the intracellular free Ca^{2+} in the pancreatic islet β-cells (Hellman 1982; Matthews & Shotton 1984). The possible mechanisms that may be responsible for this action are an ionophoretic action of these drugs or an increased net influx of Ca through voltage-operated Ca-channels. If a similar effect on intracellular free Ca were to be produced in smooth muscle cells, a contraction would be expected. Thus, the present study was undertaken to determine the effect of sulphonylureas on motility of the isolated uterus of the rat.

Basing our investigation on the fact that contractions evoked by $CaCl_2$ in smooth muscle are directly related to the influx of Ca into the cell through voltage-dependent channels (Van Breemen 1977), we have investigated the effect of tolbutamide and glibenclamide on dose-response curves to $CaCl_2$ in rat isolated K^+-depolarized uterus in Ca-free medium in order to assess whether sulphonylureas act directly on Ca entry.

The present results show that sulphonylureas act by inhibiting the contractile response to $CaCl_2$. The observation that tolbutamide and glibenclamide provoke a reduction of Emax in dose-response curves to $CaCl_2$ in depolarized medium without affecting the EC50 indicates that sulphonylureas antagonize the contractions induced by Ca in a non-competitive manner. Our results suggest that the action of sulphonylureas is not directly related to a competitive inhibition of Ca entry through voltage-dependent Ca-channels and provide a basis for the assumption that the relaxant effect of sulphonylureas may involve an additional mechanism.

This mechanism would be related to intracellular free Ca levels and in order to ascertain this, we have used a system for contraction in rat uteri that does not involve extracellular Ca (Sakai et al 1981). The maintained contraction induced by vanadate could involve inhibition of both Ca efflux and Ca accumulation through an ATP-dependent mechanism (Mironneau et al 1984), since vanadate has been described as a potent inhibitor of Ca-ATPase (Varecka & Carafoli 1982). In these conditions tolbutamide and glibenclamide are able to relax the vanadate-induced contraction. This action may be due to a reduction in the intracellular free Ca level but could also be due to a reduction in Ca action.

Both possibilities can also explain the capacity of these compounds to reduce Emax in dose-response curves to $CaCl_2$ since the effective concentrations of tolbutamide and glibenclamide in the presence or absence of external Ca were in a similar range.

We have also studied the effect of similar doses of these drugs on spontaneous contractions of rat uterus, and the fact that sulphonylureas did not modify these contractions suggests that neither drug reduced Ca action within the cell.

The apparent contradiction between the lack of effect of sulphonylureas on spontaneous activity and their inhibitory effect on contractions induced by calcium in the potassium-depolarized uterus can be explained by a sulphonylurea effect on intracellular Ca level. This occurs only when a static equilibrium is reached between the free cytosolic Ca level and stored calcium (e.g. in $CaCl_2$-induced contraction or vanadate-induced contraction), but not in the case of spontaneous contractions of the uterus.

Previous reports have shown that sulphonylureas do not penetrate into cells but act on the cell surface (Hellman 1982). The present results suggest that these drugs may act by directly promoting Ca binding to the membrane internal surface or, by indirectly relaxing smooth muscle by increasing cyclic 3,5-AMP levels (Kuo et al 1973). On the other hand, recent work indicates that the most likely mechanism of cyclic 3,5-AMP-induced relaxation is an increase in the calcium uptake by intracellular organelles (Kuriyama et al 1982).

The present results, corroborated by previous work (Villar et al 1986), question the hypothesis of the ionophoretic action of sulphonylureas, since if this hypothesis is true and these drugs behave like ionophores, they would be expected to increase the contraction of uterine smooth muscle.

REFERENCES

Basulto, J., Morcillo, E., Rubio, E., Esplugues, J. (1978) Arch. Farmacol. Toxicol. 4: 313–318

Couturier, E., Malaisse, W. J. (1980) Arch. Int. Pharmacodyn. 245: 323–334

Deleers, M., Gelbcke, M., Malaisse, W. J. (1983) FEBS Lett. 151: 260–272

Hellman, B. (1982) Acta Biol. Med. Germ. 41: 1211–1219

Henquin, J. C., Meissner, H. P. (1982) Biochem. Pharmacol. 31(7): 1407–1415

Kuo, W. N., Hodgins, D. S., Kuo, J. F. (1973) J. Biol. Chem. 248: 2705–2711

Kuriyama, H., Ito, Y., Suzuki, H., Kitamura, K., Itoh, T. (1982) Am. J. Physiol. 243: H641–H662

Matthews, E. K., Shotton, P. A. (1984) Br. J. Pharmacol. 82: 689–700

Mironneau, C., Mironneau, J., Savineau, J. P. (1984) Ibid. 82: 735–743

Sakai, K., Yamaguchi, T., Uchida, M. (1981) Arch. Int. Pharmacodyn. 250: 40–54

Van Breemen, C. (1977) J. Physiol. 272: 317–329

Van Rossum, J. M. (1963) Arch. Int. Pharmacodyn. 143: 299–330

Varecka, L., Carafoli, E. (1982) J. Biol. Chem. 257: 7414–7421

Villar, A., D'Ocon, M. P., Anselmi, E. (1986) Arch. Int. Pharmacodyn. 279: 248–257