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Short communication

Inhibition of K_{ATP} channel activity by troglitazone in CRI-G1 insulin-secreting cells

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Abstract

Patch-clamp recording techniques were used to examine the effect of troglitazone on K_{ATP} channel activity in Cambridge rat insulinoma-G1 (CRI-G1) insulin-secreting cells. In both inside-out and outside-out patch recordings, bath application of troglitazone reduced K_{ATP} channel activity. This inhibition was independent of the membrane voltage and was poorly reversible. In whole-cell studies, troglitazone inhibited K_{ATP} channel currents with an IC_{50} of 697 ± 92 nM and an associated Hill coefficient of 1.2 ± 0.2. In current clamp recordings 10 μM troglitazone depolarised the CRI-G1 cell membrane by 36.8 ± 3.9 mV with a concomitant decrease in membrane conductance. However, in contrast to the rapid depolarisation produced by tolbutamide, the effects of troglitazone developed more slowly, usually taking 15–20 min to develop.

Keywords: Troglitazone; K_{ATP} channel; β Cell; Insulin secretion

1. Introduction

The thiazolidinedione derivative, troglitazone ((±)-5-[4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl-methoxy) benzyl]-2,4-thiazolidinedione), is a recently developed orally active hypoglycaemic compound which improves insulin resistance in a number of animal models (Fujiwara et al., 1988). Several studies have demonstrated that this compound may achieve its therapeutic effects by enhancing insulin action in peripheral tissues such as skeletal muscle, liver and adipose tissue. This is thought to arise via a number of cellular actions including increased expression of the glucose transporters GLUT1 and GLUT4 and the facilitation of glucokinase gene expression (see Whitcomb and Saltiel, 1995).

In vivo studies indicate that this agent decreases plasma glucose, insulin and triglyceride levels (Fujiwara et al., 1988). In contrast to conventional oral antidiabetic agents such as the sulphonylureas, troglitazone does not appear to induce hypoglycaemia in euglycaemic animals (Whitcomb and Saltiel, 1995). However, long term treatment with troglitazone has been reported to improve degranulation of the insulin granule, its synthesis in the pancreatic β cell and also to gradually increase serum insulin levels after an insulin lowering period (Fujiwara et al., 1991). Recent in vitro studies have also shown that troglitazone is capable of directly stimulating insulin secretion from the pancreatic β cell (Masuda et al., 1995). At present, the mechanism by which this effect is achieved is unknown although Masuda et al. (1995) report that troglitazone has no direct effect upon K_{ATP} channel activity in the β cell. In contrast, the structurally related thiazolidinedione englitazone has been reported to directly inhibit K_{ATP} channel activity (I.C.M. Rowe and M.L.J. Ashford, submitted for publication). In view of this latter finding, we have re-evaluated the effect of troglitazone upon K_{ATP} channel function in the CRI-G1 insulin secreting cell line.

2. Materials and methods

2.1. Electrical recording and analysis

Cells of the rat pancreatic islet cell line CRI-G1 were cultured and passaged at 2–5 day intervals as previously described (Carrington et al., 1986). This study employed both the cell-free and whole-cell configurations of the patch-clamp recording technique, as described by Hamill

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et al. (1981). Recording electrodes were pulled from borosilicate glass capillaries and when filled with electrolyte had resistances of 8–12 MΩ for isolated patch experiments, and 2–5 MΩ for whole-cell recording. Single channel events were detected using an Axopatch 1D patch-clamp amplifier and were stored on video tape. Records used for illustrative purposes were replayed into a chart recorder (Gould 2200) which filtered the data at 140 Hz. The single channel current analysis was determined off-line using a programme that incorporated a 50% threshold crossing parameter to detect events (Dempster, 1988). Data segments between 30 and 90 s duration were replayed at the recorded speed, filtered at 1.0 kHz using an 8-pole Bessel filter, and digitised at 5.0 kHz using a Data Translation 2801A interface. The average channel activity ($N_f \cdot P_o$) where N_f is the number of functional channels in the patch and P_o is the open state probability, was determined by measuring the total time spent at each unitary current level and expressed as a proportion of the total time recorded (Lee et al., 1994). Changes in $N_f \cdot P_o$ as a result of drug effects are expressed as a percentage of control. To obtain whole-cell currents, cells were clamped at a holding potential of −70 mV, and −10 mV pulses, 200 ms in duration were applied at 2 s intervals as described previously (Lee et al., 1994). In current clamp recordings, a 10 pA hyperpolarising pulse, 1 s duration was applied every 4 s in order to assess changes in cellular conductance (Kozlowski et al., 1989). All experiments were conducted at room temperature (22–25°C).

2.2. Solutions

Before use the cells were washed thoroughly with solution A which consisted of (mM): NaCl 135.0, KCl 5.0, $CaCl_2$ 1.0, $MgCl_2$ 1.0, Hepes 10.0, pH adjusted to 7.2 with NaOH. For whole-cell voltage and current-clamp studies, the cells were bathed in solution A while the pipette contained (mM): KCl 140.0, $MgCl_2$ 1.0, $CaCl_2$ 2.0, EGTA 10.0, Hepes 10.0, pH adjusted to 7.2 with KOH which resulted in free Ca^{2+} and Mg^{2+} concentrations of 20 nM and 0.65 mM respectively (solution B).

In experiments using the inside-out configuration, the pipette contained (mM): KCl 140.0, $MgCl_2$ 1.0, $CaCl_2$ 1.0, Hepes 10.0, pH adjusted to 7.2 with KOH (solution C) whilst the bath contained solution B. In experiments on outside-out patches, the bath solution was C whilst the pipette solution was B. The concentrations of free divalent cations were determined by using a program for calculating metal ion/ligand binding 'METLIG' (P. England and R. Denton, University of Bristol).

Membrane patches were continuously perfused throughout the course of the experiment by a gravity feed system at a rate of approximately 1.5 ml s^{-1}, which allowed complete solution exchange within 20 s. In whole-cell experiments the bath was perfused at 0.5 ml s^{-1} which allowed complete exchange of solution within 1 min. Tolbutamide was made up as a 100 mM stock solution in either 0.2 M KOH or 0.2 M NaOH. Troglitazone (obtained from Warner Lambert Pharmaceuticals) was made up as a 100 mM stock solution in dimethyl sulphoxide (DMSO). In control experiments 0.1% DMSO had no effect on K_{ATP} channel activity ($n = 3$). Tolbutamide and all other drugs were obtained from Sigma (Poole, Dorset).

All data in the text and figures are presented as mean values ± S.E.M. unless otherwise stated.

3. Results

3.1. Single channel studies

Recordings of single K_{ATP} channel currents from inside-out membrane patches isolated from the CRI-G1 cell line were made in symmetrical 140 mM K^+-containing solution in the presence of 0.65 mM Mg^{2+} in the internal bath solution (Fig. 1a). Under these recording conditions, K_{ATP} channel activity was identified on the basis of conductance (55.3 ± 2.3 pS ($n = 4$) in the inward direction), inward rectification and sensitivity to bath applied ATP (100 μM produced 84.3 ± 3.4% ($n = 4$) inhibition (not shown)) or the sulphonylurea tolbutamide (100 μM produced 87.2 ± 2.1% ($n = 4$) inhibition (not shown)). When applied to the intracellular bath solution, 10 μM troglitazone reversibly inhibited K_{ATP} channel activity (Fig. 1a).

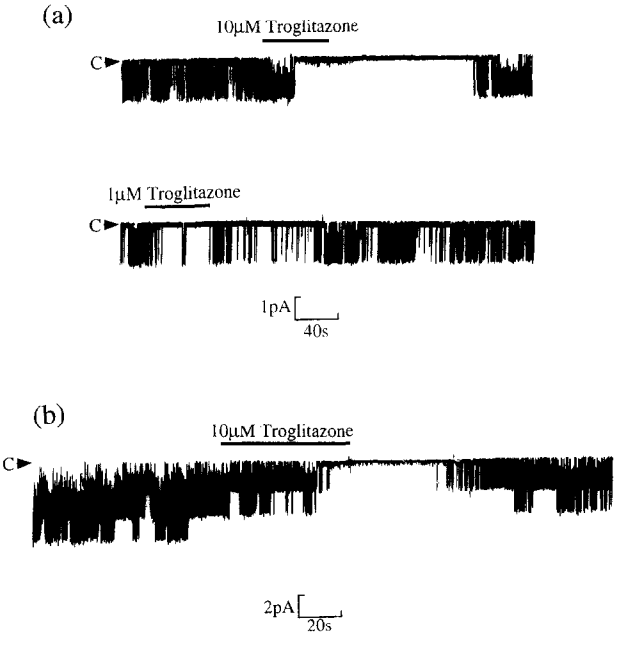

Fig. 1. Continuous single channel recordings obtained from CRI-G1 cells in symmetrical 140 mM KCl. (a) An inside-out patch held at −50 mV. Bath application of 10 μM troglitazone produced complete inhibition of K_{ATP} channel activity which was reversible on extended washing. The subsequent application of 1 μM troglitazone produced a partial inhibition of channel activity. (b) An outside-out patch held at −40 mV. In this instance the application of 10 μM troglitazone completely inhibited K_{ATP} channel activity in a reversible manner.

This inhibition of channel activity was concentration dependent, 1 μM producing 48.2 ± 3.2% ($n = 3$) inhibition, 10 μM a reduction in channel open-state probability (P_o) by 82.4 ± 4.7% ($n = 16$), and 50 μM complete channel inhibition ($n = 3$). The reduction in channel activity was not associated with a change in the single channel conductance, over the range of potentials tested (−60 mV to +60 mV), being 52.6 ± 3.2 pS ($n = 6$) in the presence of 10 μM troglitazone, nor was the inhibition of P_o induced by troglitazone voltage dependent over this range.

Since troglitazone would normally access its site of action from the external surface, a similar study was performed using the outside-out patch configuration (Fig. 1b). Once again bath-applied troglitazone inhibited K_{ATP} channel activity in a concentration dependent manner. 1 μM troglitazone inhibited K_{ATP} channel P_o by 56.0 ± 4.1% ($n = 5$), 10 μM by 88.8 ± 4.2% ($n = 11$) and 50 μM troglitazone completely inhibited K_{ATP} channel activity ($n = 4$). These actions of troglitazone were independent of membrane potential over the range +60 to −60 mV.

3.2. Whole-cell studies

Detailed studies of the concentration dependence of K_{ATP} channel inhibitors cannot easily be performed on isolated membrane patches. The principal reasons for this

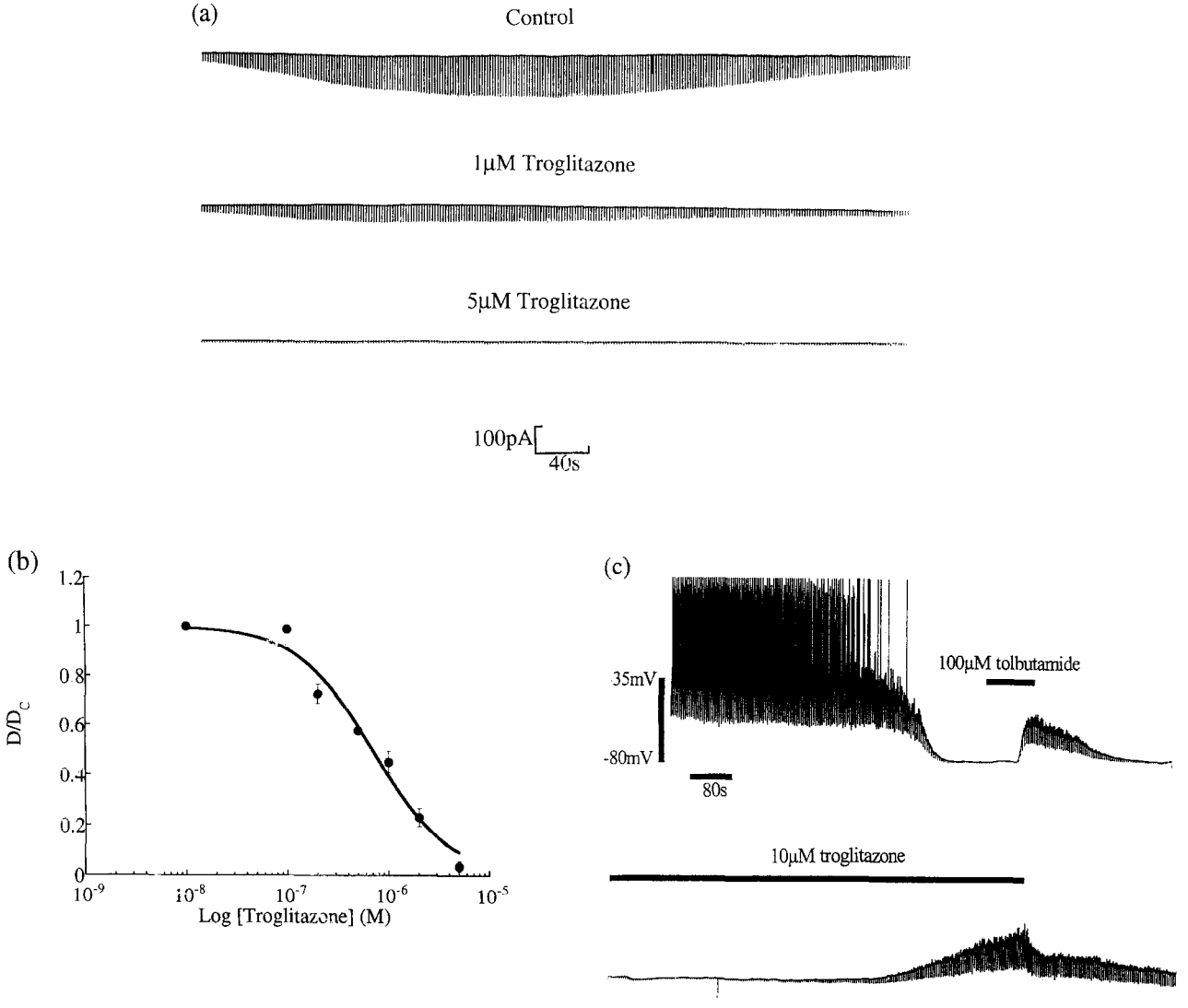

Fig. 2. (a) The effect of troglitazone on whole-cell K_{ATP} channel currents. Each trace was obtained from a separate cell of similar capacitance (15 pF). Each cell was incubated in the indicated concentration of troglitazone for 1 h prior to the onset of recording. (b) The effect of increasing concentrations of troglitazone upon the whole-cell K_{ATP} channel current density. The data were fit to the following equation: $D/D_c = 1/(1 + (a/b)^n)$ where D = whole-cell K_{ATP} channel current density in the presence of troglitazone, D_c = control whole-cell K_{ATP} channel current density, a = drug concentration, b = half maximal inhibitory concentration and n = Hill coefficient. (c) Whole-cell current clamp recording comparing the time course of depolarisation induced by tolbutamide and troglitazone. Hyperpolarising current pulses were applied throughout the course of the experiment often resulting in anode-break action potentials, though spontaneous action potentials were also evident. At the beginning of the experiment, the membrane potential was −39 mV and this gradually became more negative as the ATP dialysed out of the cell. Application of tolbutamide induced a rapid, readily reversible depolarisation. In contrast, the effect of troglitazone took much longer to develop and was much less reversible.

are the wide variations in the channel activity between patches and the phenomenon of channel rundown. A more accurate means by which to assess the concentration dependence of the above compounds is to use the whole-cell configuration where the phenomenon of rundown is continuous and can be quantified (Trube et al., 1986) allowing drugs to be tested over a wide range of concentrations.

Under whole-cell voltage clamp conditions, concentrations of troglitazone up to 10 μM were found to have little effect during the initial 4–5 min of application and removal of the drug after longer periods was poorly reversible. Thus using this approach, it was difficult to distinguish between K_{ATP} channel current inhibition and rundown. To overcome this problem, the experimental protocol established by Zunkler et al. (1988) was employed. Cells were incubated for 1–2 h with varying concentrations of troglitazone added to the extracellular solution. Thereafter, whole-cell K_{ATP} channel currents were measured as described in the methods and the peak whole-cell K_{ATP} channel current determined under each condition. Differences in cell size were taken into account by dividing the maximum current amplitude measured from each cell by its measured capacitance (Zunkler et al., 1988) which was estimated by adjusting the capacity compensation network of the patch clamp amplifier. Fig. 2a demonstrates the typical characteristics of the whole-cell K_{ATP} channel currents measured under these conditions. A current density of 14.2 ± 1.3 pA/pF ($n = 25$) was found under control conditions. When cells were preincubated with 100 μM or 500 μM tolbutamide, $89.2 \pm 2.3\%$ ($n = 5$) and $97.6 \pm 1.2\%$ ($n = 5$) inhibition of the peak whole-cell current density respectively was observed thus confirming that this current is due to K_{ATP} channel activation. Using this approach, troglitazone was found to inhibit the peak whole-cell current density in a concentration dependent manner (Fig. 2a) with an IC_{50} of 697.1 ± 92.5 nM (73 cells) and an associated Hill coefficient of 1.2 ± 0.2 (Fig. 2b).

The results presented so far indicate that troglitazone is able to inhibit K_{ATP} channel activity in CRI-G1 insulin secreting cells. In order to establish the effects of this compound upon membrane potential, recording was performed under current clamp conditions. Following rupture of the cell membrane, the initial resting membrane potential in the presence of 10 mM glucose in the extracellular solution was -44.5 ± 4.8 mV ($n = 12$). This became more negative before stabilising at a value of -71.8 ± 1.0 mV ($n = 12$). Application of 100 μM tolbutamide induced a rapid depolarisation of 26.7 ± 5.0 mV ($n = 6$) which was associated with a decrease in whole-cell conductance (Fig. 2c). As observed with tolbutamide, the application of 10 μM troglitazone was found to produce a depolarisation of 36.8 ± 3.9 mV ($n = 8$) with concomitant decrease in membrane conductance. However, in contrast to the rapid depolarisation produced by tolbutamide, the effects of troglitazone developed much more slowly, usually taking 15–20 min to develop. These effects were also poorly reversible under the present experimental conditions (Fig. 2c).

4. Discussion

In the present study it is clearly shown that the antidiabetic compound troglitazone is able to inhibit K_{ATP} channel activity in the CRI-G1 insulin secreting cell line. These findings are in direct contrast to those reported recently by Masuda et al. (1995). However, upon inspection of the results presented by Masuda et al., it appears that troglitazone was tested for an insufficient period of time (3 min). In the present study, we found that in order to achieve maximal effectiveness, at least 20 min application was required in whole-cell studies. The reasons for this are not clear but this situation resembles that seen with the second generation sulphonylureas such as glibenclamide which have effects that are also slow in onset and poorly reversible (Zunkler et al., 1988). The mechanism by which troglitazone achieves its inhibitory effects are uncertain. Masuda et al. (1995) demonstrate that this compound can interact with the sulphonylurea receptor in radioligand binding studies and it will be interesting to examine if this compound mediates its K_{ATP} channel inhibitory effects via this receptor.

The concentration of troglitazone present in the plasma of patients undergoing treatment is thought to be in the μM range (Masuda et al., 1995). From the present study such concentrations would be expected to interfere with K_{ATP} channel activity. However, although troglitazone, like the sulphonylureas is able to inhibit K_{ATP} channel activity at therapeutic concentrations, this compound differs from conventional antidiabetic compounds in that most in vivo and clinical studies report that it appears to be devoid of hypoglycaemic side effects. Indeed, studies have demonstrated that troglitazone can actually reduce the level of circulating insulin (Fujiwara et al., 1988). Consequently, it is difficult to put the findings of the present study into context. Masuda et al. (1995) suggest that any insulinotropic effect of troglitazone might be overwhelmed by the consequences of decreasing insulin resistance resulting in reduced plasma insulin levels. However, it is not presently known whether this compound is able to stimulate insulin secretion in vivo, nor is it known whether this effect is important to its therapeutic action.

In future studies it will be interesting to examine the ability of troglitazone to interact with K_{ATP} channels in other tissues such as cardiac myocytes (Noma, 1983). Furthermore, it will be interesting to examine this compound's effect on other types of ion channel in order to establish its selectivity for the K_{ATP} channel. In relation to this it has been reported that the related thiazolidinediones ciglitazone and pioglitazone are able to reduce blood pressure in various animal models by a mechanism apparently involving a decrease in calcium entry (Pershadsingh et al., 1993; Zhang et al., 1994).

References

Carrington, C.A., E.D. Rubery, E.C. Pearson and C.N. Hales, 1986, Five new insulin-producing cell lines with differing secretory properties, J. Endocrinol. 109, 193–200.

Dempster, J., 1988, Computer analysis of electrophysiological signals, in: Microcomputers in Physiology, A Practical Approach, ed. P.J. Fraser (IRL Press, Oxford) p. 51–95.

Fujiwara, T., S. Yoshioka, T., Yoshioka, I. Ushiyama and H. Horikoshi, 1988, Characterisation of new oral antidiabetic agent CS-045, Diabetes 37, 1549–1558.

Fujiwara, T., M. Wada, K. Fukuda, M. Fukami, S. Yoshioka, T. Yoshioka and H. Horikoshi, 1991, Characterisation of CS-045, a new oral antidiabetic agent, II. Effects on glycemic control and pancreatic islet structure at a late stage of the diabetic syndrome in C57BL/KsJ-db/db mice, Metabolism 40, 1213–1218.

Hamill, O.P., A. Marty, E. Neher, B. Sakmann and F.J. Sigworth, 1981, Improved patch clamp techniques for high resolution current recording from cells and cell free membrane patches, Pflügers Arch. 391, 85–100.

Kozlowski, R.Z., C.N. Hales and M.L.J. Ashford, 1989, Dual effects of diazoxide on ATP-K^+ currents recorded from an insulin-secreting cell line, Br. J. Pharmacol. 97, 1039–1050.

Lee, K., S.E. Ozanne, C.N. Hales and M.L.J. Ashford, 1994, Mg^{2+} dependent inhibition of K_{ATP} channels by sulphonylureas in CRI-G1 insulin secreting cells, Br. J. Pharmacol. 111, 632–640.

Masuda, K., Y. Okamoto, Y. Tsuura, S. Kato, T. Miura, K. Tsuda, H. Horikoshi, H. Ishida and Y. Seino, 1995, Effects of troglitazone (CS-045) on insulin secretion in isolated rat pancreatic islets and HIT cells: an insulinotropic mechanism distinct from glibenclamide, Diabetologia 38, 24–30.

Noma, A., 1983, ATP-regulated K^+ channels in cardiac muscle, Nature 305, 147–148.

Pershadsingh, H.A., J. Szollosi, S, Benson, W.C. Hyun, B.G. Feuerstein and T.W. Kurtz, 1993, Effects of ciglitazone on blood pressure and intracellular calcium metabolism, Hypertension 21, 1020–1023.

Trube, G., P. Rorsman and T. Ohno shosaku, 1986, Opposite effects of tolbutamide and diazoxide on the ATP dependent K^+ channel in mouse pancreatic β-cells, Pflügers Arch. 407, 493–499.

Whitcomb, R.W. and A.R. Saltiel, 1995, Thiazolidinediones, Exp. Opin. Invest. Drugs 4, 1299–1309.

Zhang, H.-Y., S.R. Reddt and T.A. Kotchen, 1994, Antihypertensive effect of pioglitazone is not invariably associated with increased insulin sensitivity, Hypertension 24, 106–110.

Zunkler, B.J., S. Lenzen, K. Manner, U. Panten and G. Trube, 1988, Concentration-dependent effects of tolbutamide, meglitinide, glipizide, glibenclamide and diazoxide on ATP-regulated K^+ currents in pancreatic B-cells, Naunyn-Schmiedebergs Arch. Pharmacol. 337, 225–230.