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Pharmacological actions of ifenprodil in the rat isolated anococcygeus muscle

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The effect of ifenprodil on the contractile responses of the rat anococcygeus muscle to noradrenaline, methoxamine, carbachol, potassium chloride and calcium chloride has been examined. Ifenprodil (10^{-8}–10^{-7} M) non-competitively antagonized noradrenaline and methoxamine-induced contractions while carbachol-induced responses were unaffected. Similarly, contractile responses to KCl (in tissues from reserpinized animals, or tissues bathed in Tyrode containing 10^{-6} M phentolamine) and $CaCl_2$ in tissues bathed with Ca^{2+}-free depolarizing solution were blocked non-competitively by ifenprodil (10^{-7}–10^{-6} M). These findings suggest that ifenprodil possesses α-adrenoceptor antagonist activity, with intrinsic Ca^{2+} blocking properties.

Ifenprodil, (±)-2-(4-benzylpiperidino)-1-(4-hydroxyphenyl)propan-1-ol tartrate monohydrate) is the most active of a series of 1-(substituted phenyl)-2-piperidino-alkanol derivatives synthesized by Carron et al (1971). According to these authors, the compound exhibits α-adrenoceptor blocking property in the dog perfused hind limb and also in the rat seminal vesicle. The α-adrenoceptor blocking property of ifenprodil has been further demonstrated in both anaesthetized normal and pithed rats (Cavero & Lefevre-Borg 1981) and also in the isolated vas deferens of rabbits and rats, as well as in rabbit aortic ring preparations (Casadamont et al (1981). Ifenprodil was shown in the latter study to block both α_1- and α_2-adrenoceptor subtypes over similar concentration range.

However, in a recent study in the perfused mesenteric artery of the rat, ifenprodil was observed to block vascular vasoconstriction induced by α-adrenoceptor agonists (noradrenaline and cirazoline) and also those evoked by high potassium depolarization and calcium chloride (Adeagbo 1984). It has thus been suggested that ifenprodil blocks Ca^{2+} mobilization through both the channels coupled to α-adrenoceptors and the potential sensitive channels. The present study examined the effect of ifenprodil in the rat isolated anococcygeus muscle.

Materials and methods

Male albino rats (250–300 g) were killed by a blow on the head and exsanguinated. The abdomen was opened and the paired anococcygeus muscles isolated as described by Gillespie (1972). Each muscle was set up under a resting tension of 0·75 g in a 10 ml organ bath containing Tyrode solution at 37 °C bubbled continuously with air. The tissue was allowed to equilibrate for 1 h during which the Tyrode solution in the bath was routinely replaced every 15–20 min. Agonist-induced contractions magnified 6-fold were recorded isotonically on smoked paper. In all cases, agonists were added non-cumulatively to the bath and a tissue-drug contact time of 90 s was maintained. Tissues were allowed a 5 min resting period between contractions. The highest contractile response attainable to each agonist in each tissue was taken as 100% and all submaximal effects were calculated as a percentage of this value.

Ifenprodil was added to the bath in Tyrode solution and the tissue was allowed to equilibrate with the antagonist for 30 min before the agonist concentration–response curve was re-established. Ifenprodil was assessed for its receptor blocking activity by using it in the presence of α-adrenoceptor agonists, noradrenaline and methoxamine, and the cholinergic receptor agonist, carbachol. Experiments designed to assess the non-specific effects of ifenprodil were of two types. First, ifenprodil was used against KCl (as agonist) in rats pretreated with reserpine (4 mg kg^{-1} i.p.) 18–24 h before death; or sometimes in muscles from untreated rats in the presence of 10^{-6} M phentolamine. In the second type of experiment, the effect of ifenprodil on $CaCl_2$-induced contractions in Ca^{2+}-free depolarizing Tyrode solution (plus 10^{-6} M phentolamine) was examined.

Statistical analysis. Results are expressed as mean ± s.e.m. Statistical difference was obtained with Student's *t*-test and the difference between mean values were considered significant when $P < 0.05$.

Solutions and drugs. The Tyrode solution had the following composition (mM): NaCl 137, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 0·9, NaH_2PO_4 0·3, $NaHCO_3$ 11·9, and glucose 5·6. The composition of Ca^{2+}-free, high K^+ depolarizing Tyrode solution was (mM): NaCl 84·7, KCl 55, NaH_2PO_4 0·3, $MgCl_2$ 0·9, $NaHCO_3$ 11·9 and glucose 5·6. The drugs used were: ifenprodil tartrate monohydrate (Synthelabo, France), carbamylcholine chloride (carbachol) and (−)-noradrenaline bitartrate (Sigma), (±)-methoxamine hydrochloride (Burroughs Wellcome), phentolamine hydrochloride (Ciba Geigy),

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potassium chloride, and calcium chloride (BDH Chemicals Ltd); Reserpine (Serpasil Ciba-Geigy).

Results

Effect of ifenprodil on contractions induced by noradrenaline, methoxamine and carbachol. Noradrenaline ($3 \cdot 0 \times 10^{-7}$–$1 \cdot 9 \times 10^{-5}$ M), methoxamine ($2 \cdot 0 \times 10^{-7}$–$1 \cdot 3 \times 10^{-5}$ M) and carbachol ($1 \cdot 4 \times 10^{-6}$–$4 \cdot 4 \times 10^{-5}$ M) each induced reproducible concentration-dependent contractions of the rat anococcygeus muscle. The contractions were rapid in onset in all cases and the tension of the contraction was maintained until the agonist was washed out of the bath. Ifenprodil (up to 10^{-5} M) did not modify the resting tone of the muscle, but over the range 10^{-8}–10^{-7} M antagonized, in a concentration-related fashion, the concentration-response curves to noradrenaline and methoxamine causing a rightward non-competitive shift (Fig. 1). The concentration-response curve to carbachol was not significantly affected by ifenprodil (10^{-5} M) suggesting that ifenprodil does not block cholinoceptors and may be selective for α-adrenoceptors or α-adrenoceptor-mediated events. In all cases, however, the responses to noradrenaline or methoxamine were restored to control values after about 1 h of washing out ifenprodil, ruling out the possibility of antagonist-induced desensitization of α-adrenoceptors in the tissue.

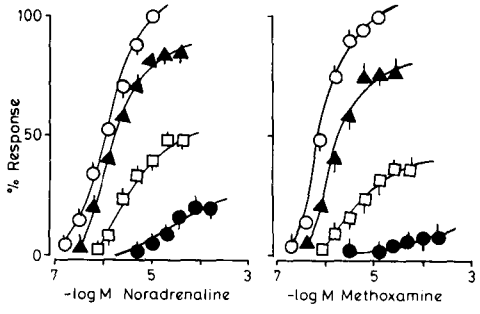

FIG. 1. Effect of ifenprodil on the concentration–response curves of the rat anococcygeus muscle to noradrenaline (left) and methoxamine (right). (○) control responses, while (▲), (□) and (●) are responses in the presence of 10^{-8}, 5×10^{-8} and 10^{-7} M ifenprodil, respectively. The agonists were tested in separate tissue preparations and each point on the graph represents the mean (±s.e.m.) of 8 experiments.

Effect of ifenprodil on the contractile responses induced to KCl and $CaCl_2$. The influence of ifenprodil on KCl-induced contractile responses was examined to test whether its antagonism of noradrenaline was specific for α-adrenoceptors or was accompanied by some non-specific components. Since part of the contractile response to KCl in the anococcygeus muscle involves the release of noradrenaline from intramural nerve endings (Gibson & Pollock 1973), the interaction between ifenprodil and KCl was studied on reserpinized rats. KCl ($1 \cdot 3 \times 10^{-3}$–$1 \cdot 3 \times 10^{-1}$ M) consistently and concentration-dependently contracted the muscle and ifenprodil (10^{-7}–10^{-6} M) concentration dependently antagonized the contractions. All the concentrations of ifenprodil tested suppressed the maximum response to KCl in this tissue (Fig. 2a).

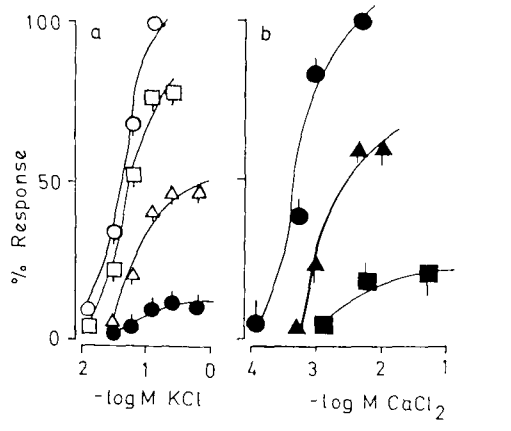

FIG. 2(a). Effect of ifenprodil on the concentration–response curves of KCl in the rat anococcygeus muscle. (○) control responses, while (□), (△) and (●) are responses in the presence of 10^{-7}, 2×10^{-7} and 10^{-6} M ifenprodil, respectively. Each point on the graph represents a mean (±s.e.m.) of 8 experiments and (b) Concentration–response curves of the rat anococcygeus muscle to $CaCl_2$ in Ca^{2+}-free depolarizing Tyrode solution. (●) control responses, while (▲) and (■) are responses in the presence of $5 \cdot 0 \times 10^{-7}$ and 10^{-6} M ifenprodil, respectively. Each point represents the mean (±s.e.m.) of 8 experiments.

Similarly, rat anococcygeus muscle equilibrated in Ca^{2+}-free K^+ depolarizing medium (also containing 10^{-6} M phentolamine), concentration-dependently contracted to sequential additions of $CaCl_2$ ($1 \cdot 0 \times 10^{-4}$–$5 \cdot 0 \times 10^{-3}$ M). Concentration-response curves obtained to $CaCl_2$ at 30 min intervals were similar. Ifenprodil ($5 \cdot 0 \times 10^{-7}$ and 10^{-6} M) antagonized Ca^{2+}-induced contractile responses with a marked suppression of maximal responses (Fig. 2b).

Discussion

The present results show that ifenprodil blocks α-adrenoceptors or α-adrenoceptor mediated events in smooth muscle excitation–contraction coupling. This is evident because ifenprodil selectively though non-competitively blocked α-adrenoceptor mediated contractions induced by noradrenaline and methoxamine, while contractile responses induced by carbachol were unaffected. The α-adrenoceptor blocking property observed is consistent with previous findings in vascular and non-vascular smooth muscle preparations (Carron et al 1971; Cavero

& Lefevre-Borg 1981; Casadamont et al 1981). Non-competitive blockade of α-adrenoceptors could occur if ifenprodil interacts irreversibly with the receptor site to prevent agonist binding. This seems unlikely since responses to noradrenaline and methoxamine were fully restored to control values following short-term washout of the antagonist.

Oriowo (1982) showed that noradrenaline and carbachol (stimulating α- and muscarinic receptors, respectively) failed to contract the rat anococcygeus muscle in the absence of extracellular Ca^{2+} implying that a transmembranal Ca^{2+} influx is involved in the contractile responses induced by both agonists. Yet ifenprodil interacts with these agonists differently. This in essence would suggest that even though sources of Ca^{2+} for contraction by a number of agonists may be the same, the pathway (or ionic channels) through which different pharmacological receptor-stimulating agonists promote Ca^{2+} influx across the cell membrane might be different. The fact that ifenprodil did not affect carbachol-induced contractions may suggest that the ionic pathways coupled to α-adrenoceptors and the muscarinic receptors in the anococcygeus muscle may be different. It would appear that ifenprodil selectively blocked the ionic pathways coupled to α-adrenoceptors such that it prevents the influx of Ca^{2+} through the α-receptor operated channels. Bolton (1979) proposed the existence of qualitatively similar ionic channels for pharmacological receptors.

Besides receptor mediated actions, ifenprodil was assessed for 'calcium antagonistic' property. This is rational because ifenprodil was developed as a vasodilator (Carron et al 1971) and most vasodilators are known to interfere with calcium fluxes across the cell membrane, e.g. the non-specific calcium antagonists like cinnarizine, chlorpromazine, papaverine and local anaesthetics (Godfraind & Kaba 1972) and the specific slow channel calcium antagonists—nifedipine and verapamil (Fleckenstein 1977). Ifenprodil, at all concentrations tested, antagonized contractions to KCl. Contraction of the rat anococcygeus muscle to KCl comprises two components (Gibson & Pollock 1973): an indirect action through the release of noradrenaline and a direct depolarizing action on the smooth muscle cell membrane. Ifenprodil antagonism of KCl was not due to inhibition of the indirect α-adrenoceptor action of KCl since the antagonism was obtained in muscles taken from animals pretreated with reserpine to deplete noradrenaline stores in the tissues, and the antagonism also occurred in tissues bathed in Tyrode solution containing 10^{-6} M phentolamine to block the α-adrenoceptors. On the other hand, ifenprodil may be acting as a non-specific blocker of trans-membranal influx of calcium across the smooth muscle cell membrane through the so-called potential sensitive channels, since KCl contractions mediated by direct depolarization of smooth muscle membrane is highly dependent upon the presence of extracellular Ca^{2+}. This explanation is further enhanced by the observation that ifenprodil antagonized calcium-induced contractions in Ca^{2+}-free depolarizing medium since Ca^{2+} contractions in depolarizing media has been attributed to extracellular Ca^{2+} influx in vascular (Godfraind & Kaba 1969; Van Breemen 1977) and non-vascular (Spedding 1982) smooth muscle preparations.

It may be concluded that ifenprodil is an α-adrenoceptor blocker with intrinsic Ca^{2+} antagonistic properties.

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