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Prejunctional α_2-Adrenoceptor Mediated Inhibitory Action of Clonidine and B-HT 920, but Not Urapidil in Guinea Pig Ileum[1] (41994)

RONALD J. SHEBUSKI AND BEN G. ZIMMERMAN

Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455

Abstract. Isolated guinea pig ileal longitudinal muscle was stimulated transmurally with a frequency of 0.1 Hz, duration of 0.5 msec, and supramaximal voltage (80–100 V). Transmural stimulation induces ileal contractions via activation of cholinergic neurons. α_2-Adrenergic agonists block the response to transmural stimulation via activation of prejunctional α_2 receptors which inhibit release of acetylcholine from cholinergic nerve terminals. Urapidil has been reported to have α_2-agonistic actions, and therefore was compared to the prototypic α_2 agonists, clonidine and B-HT 920. Clonidine and B-HT 920 depressed responses to transmural stimulation in the guinea pig ileum. Clonidine was the most potent inhibitor of the contractions, followed closely by B-HT 920. Very high concentrations of urapidil were necessary to suppress nerve-induced contractions of the ileum. The effects of clonidine and B-HT 920, but not urapidil, were antagonized by the selective α_2 antagonist, yohimbine. In unstimulated preparations, in which exogenous acetylcholine was used to elicit contractions of the ileum, urapidil depressed the response while clonidine and B-HT 920 had no effect. When $PGF_{1\alpha}$ was used to contract the ileum, no inhibitory effects were noted for urapidil, clonidine, or B-HT 920. Therefore urapidil, only in high concentrations, inhibits the contraction to transmural stimulation by depressing the response at a postjunctional cholinergic site. No evidence was found that urapidil can act as an agonist at a prejunctional α_2-receptor site. © 1985 Society for Experimental Biology and Medicine.

Urapidil is a phenylpiperazine substituted uracil derivative which possesses both peripheral α_1- and α_2-adrenoceptor antagonistic properties (1, 2), as well as centrally induced cardiovascular actions (3). It was also reported that urapidil acted as a partial α_2 agonist at a prejunctional site in the rat vas deferens to inhibit the twitch response (1). Therefore a clonidine-like action was proposed to contribute to the mechanism of the cardiovascular effects of this compound.

In order to investigate whether or not urapidil was acting like clonidine, which inhibits norepinephrine and acetylcholine release by a prejunctional α_2-agonist action, the transmurally stimulated guinea pig ileum preparation was utilized. Stimulation by norepinephrine of prejunctional α-adrenoceptors on cholinergic nerve terminals leads to a decrease in acetylcholine release in a similar manner as this mechanism operates to inhibit norepinephrine release from adrenergic nerves (4, 5). Therefore activation of prejunctional α_2-adrenoceptors on cholinergic nerve terminals reduces contractile responses of guinea pig ileum to nerve stimulation. Prejunctional α_2-adrenoceptors in the guinea pig ileum are assumed to be the same type as those located prejunctionally in sympathetically innervated tissues (6). Therefore, we considered this to be a good model for assessing α_2-agonistic properties of test compounds.

Clonidine has been demonstrated to be a potent α_2 agonist in the guinea pig ileum, reducing contractions of transmurally stimulated preparations markedly (6). In this study, clonidine and B-HT 920, another potent α_2 agonist (7), were compared to urapidil on responses of guinea pig ileum to transmural nerve stimulation. Yohimbine is an α_2 antagonist which inhibits the effects of α_2 agonists on the ileum (6). Therefore, blockade of the inhibitory effects of α_2 agonists by yohimbine serves as further evidence for a prejunctional site of action.

Materials and Methods. *Preparation of transmurally stimulated guinea pig ileum.* Guinea pigs of either sex (Bio-Labs, White Bear Lake, Minn.) weighing 300 to 400 g

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were killed by cervical dislocation and the small intestine was removed. Segments of ileum 2 to 3 cm long were obtained, discarding the 10 cm in closest proximity to the ileocaecal junction (6). The segments were placed in Krebs-bicarbonate solution which contained the following (in mM): NaCl, 118; $NaHCO_3$, 25; KCL, 4.7; Mg_2SO_4, 0.6; $CaNa_2$–EDTA, 0.026; $CaCl_2$ 2.5; KH_2PO_4, 1.2; and glucose, 11. The Krebs solution was maintained at 37°C and contained cocaine (10 μM) to block norepinephrine uptake and propranolol (1 μM) and chlorpheniramine (1.2 μM) to block β receptors and histamine receptors, respectively. The longitudinal muscle layer was removed by sliding the ileum onto a small glass rod and stripping the mesentery. By stroking away from the mesenteric border, the longitudinal muscle sheath was removed from the underlying circular muscle (8). The ileal longitudinal muscle layer was then tied at both ends and suspended from an isometric transducer (Statham UC3) at an initial tension of 1 g in a 10- to 20-ml tissue bath. The tissues were continuously aerated with 95% O_2 and 5% CO_2. The muscles were mounted in the bath between two platinum electrodes so that the nerve terminals could be stimulated transmurally. Supramaximal voltage was determined 1 hr after an initial equilibration period and this voltage was utilized for that particular experiment. The preparation was stimulated at a frequency of 0.1 Hz and a duration of 0.5 msec. Contractions due to transmural nerve stimulation were recorded on a Gilson polygraph.

Determination of dissociation constants for yohimbine in transmurally stimulated guinea pig ileum. pA_2 values, which represent the negative logarithm of the K_B (K_B is the dissociation constant for the antagonist–receptor interaction), were determined for yohimbine according to Schild (9) utilizing clonidine and B-HT 920 as selective α_2 agonists. Yohimbine was incubated in the bath at concentrations ranging from 28 nM to 2.8 μM for 60 min and then dose ratios were determined for each agonist at each concentration of antagonist tested. Dose ratios represent the ED_{50} of the agonist in the presence of the antagonist (yohimbine) divided by the ED_{50} in the absence of antagonist. Agonists were administered in the control period until a stable and reproducible response was obtained. Concentration–response curves for each agonist were constructed in a cumulative manner according to the technique of van Rossum (10). A plot of the logarithm of the dose ratio − 1 versus the negative logarithm of the yohimbine molar concentration will yield a straight line with a value of x at $y = 0$ equal to the $-\log K_B$. Schild plots are not only useful for determination of dissociation constants for antagonists but also differentiate competitive from noncompetitive antagonism. If the slope of the line is not significantly different from one, the antagonism can be judged to be competitive in nature (11). pD_2 values (negative logarithm of the ED_{50}) were also determined for clonidine, B-HT 920, and urapidil by plotting the percentage inhibition of the twitch response versus the negative logarithm of the molar concentration of the agonist. The maximal inhibition obtainable with clonidine, B-HT 920, and urapidil was 75–85%. Potential differences in maximal response were not considered when calculating ED_{50} values for clonidine, B-HT 920, or urapidil.

Statistical analysis. All results are expressed as the means ± SEM. Student's t test was used to determine statistical significance ($P < 0.05$) of the difference between two means. Linear regression analysis was applied to construct concentration–response curves and statistical differences between curves were established by comparison of ED_{50} values.

Substances used. All drugs were prepared daily in 0.9% saline and kept on ice throughout the experiment. Urapidil was supplied by Marion Laboratories Inc. (Kansas City, Mo.). B-HT 920 and clonidine hydrochloride were gifts from Boehringer Ingelheim Ltd. Other substances used were yohimbine (K and K Laboratories, Plainview, N.Y.); cocaine hydrochloride (Merck, Sharp and Dohme, West Point, Pa.); chlorpheniramine (Schering, Bloomfield, N.J.); propranolol (Sigma Chemical Co., St. Louis, Mo.); and $PGF_{1\alpha}$ (Upjohn Co., Kalamazoo, Mich.).

Results. Concentration–response curves for clonidine, B-HT 920, and urapidil in the presence and absence of 2.8 μM yohimbine are plotted in Fig. 1. A statistically significant parallel shift in the concentration–response

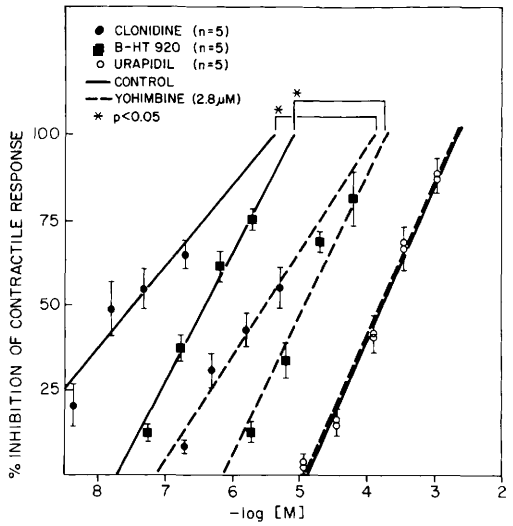

FIG. 1. Concentration-response characteristics for clonidine, B-HT 920, and urapidil in the presence and absence of yohimbine (2.8 μM). Concentration-response curves for clonidine, B-HT 920, and urapidil are plotted as the percentage inhibition of the contractile response versus the negative logarithm of the molar concentration of the agonists. Clonidine was the most potent inhibitor of the response, followed by B-HT 920 and then urapidil. The curves for clonidine and B-HT 920 are shifted significantly to the right by yohimbine, a selective α_2 antagonist, whereas that for urapidil was unaffected by yohimbine pretreatment.

curves for clonidine and B-HT 920 occurred in the presence of yohimbine. The shift to the right produced by 2.8 μM yohimbine was 89.1-fold for clonidine and 28.2-fold for B-HT 920. The concentration-response curve for urapidil was unchanged in the presence of 2.8 μM yohimbine.

Schild plots were constructed for yohimbine against clonidine and B-HT 920 in order to determine if both these compounds stimulated α_2 receptors. Figure 2 illustrates that clonidine and B-HT 920 are competitively antagonized by yohimbine with slopes and corresponding 95% confidence intervals of 1.06 (0.92–1.20) and 0.87 (0.73–1.01), respectively. These slopes are not statistically different from a theoretical Schild value of one (11), indicating competitive antagonism by yohimbine. The $-\log K_B$ values for yohimbine versus clonidine and B-HT 920 were 7.58 and 7.14, respectively. The $-\log K_B$ value for yohimbine with clonidine as an agonist (7.58) is in good agreement with the value of 7.78 reported by Drew (6). A Schild plot for urapidil could not be constructed as the effects of urapidil were not antagonized by yohimbine.

Table I indicates the ED_{50} and corresponding pD_2 values for clonidine, B-HT 920, and urapidil on transmurally stimulated guinea pig ileum. The order of potency as inhibitors of the contractions is clonidine > B-HT 920 $\gg$ urapidil. The pD_2 value of 7.45 calculated for clonidine is in agreement with the value of 7.70 reported by Wikberg (12). Very high concentrations of urapidil as compared to clonidine and B-HT 920 are necessary to suppress the contractions due to nerve stimulation.

Due to the fact that urapidil is believed to possess both α_1- and α_2-antagonistic properties (1, 2), we tested its ability to antagonize

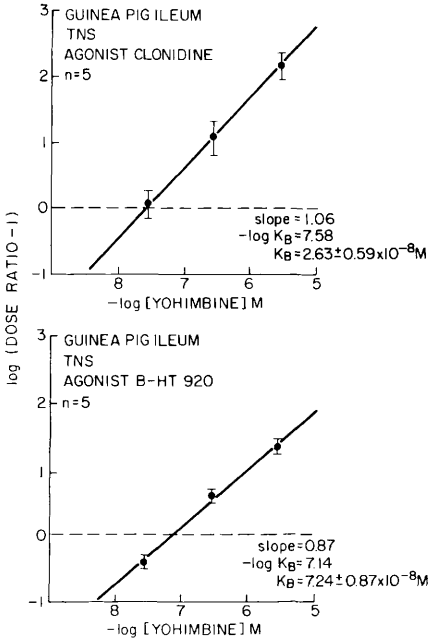

FIG. 2. Schild plots for yohimbine as an antagonist of the clonidine and B-HT 920 induced inhibition of the twitch response in transmural nerve stimulated (TNS) guinea pig ileum. Top panel: clonidine is employed as the agonist with a resulting slope of 1.06 and the $-\log K_B$ value of yohimbine equalled 7.58. Bottom panel: B-HT 920 as agonist results in a slope of 0.87 and $-\log K_B$ value of 7.14. These data indicate that both clonidine and B-HT 920 stimulate similar receptor subtypes (α_2) and are competitively antagonized by yohimbine.

TABLE I. CHARACTERISTICS OF CLONIDINE, B-HT 920, AND URAPIDIL ON INHIBITION OF THE TWITCH RESPONSE IN TRANSMURALLY STIMULATED GUINEA PIG ILEUM

Compound	N	ED_{50} (M)	pD_2 ($-\log ED_{50}$)	Dose ratio of yohimbine[a]
Clonidine	5	3.5×10^{-8}	7.45	89.1
B-HT 920	5	4.0×10^{-7}	6.38	28.2
Urapidil	5	1.7×10^{-4}	3.78	1.0

[a] ED_{50} in presence of yohimbine (2.8 μM) divided by control ED_{50}.

the effects of clonidine in three experiments on the guinea pig ileum. Urapidil was incubated in the bath for 1 hr in concentrations that suppressed ileal contractions 3–30% (2.6–26 μM) and then ED_{50} values were determined for clonidine. The clonidine ED_{50} values were either unchanged or actually decreased in the presence of urapidil indicating that urapidil, in these concentrations, had no prejunctional α_2-antagonistic action. When higher concentrations of urapidil were utilized (>26 μM), contractions were suppressed markedly thus preventing determination of ED_{50} values for clonidine.

If urapidil is not acting via prejunctional α receptors, another mechanism must be responsible for the ability of high concentrations of this agent to reduce contractions of transmurally stimulated ileum. Therefore, ileal strips were prepared that were not transmurally stimulated to elicit contractions. Instead, acetylcholine was added to the bath at concentrations of 1 and 10 ng/ml to induce contractions directly. Figure 3 illustrates the effects of urapidil, clonidine, and B-HT 920 on acetylcholine-induced contractions. Urapidil suppressed the contractions due to exogenous acetylcholine significantly at both concentrations tested. Concentrations of urapidil used were the ED_{50} and ED_{75} concentrations as determined previously on transmurally stimulated preparations. Clonidine and B-HT 920 had no effect on contractions induced by acetylcholine when tested at their ED_{50} concentrations.

Contractions were also elicited in unstimulated ileal preparations by addition of 1 and 10 ng/ml $PGF_{1\alpha}$ (n = 2). Neither urapidil, clonidine, nor B-HT 920 had any effect on these responses when tested at their ED_{50} and ED_{75} concentrations. Therefore, it would appear that urapidil, in high concentrations, blocks cholinergic receptors at a postjunctional site of action to account for suppression of ileal contractions in transmurally stimulated preparations.

Discussion. This study confirms the potent prejunctional α_2-adrenoceptor mediated in-

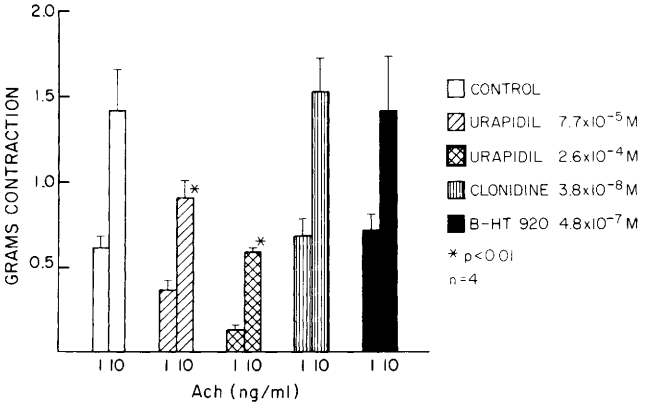

FIG. 3. Comparison of urapidil, clonidine, and B-HT 920 on the direct contractile response of unstimulated guinea pig ileum to exogenous acetylcholine. Urapidil, when tested at ED_{50} and ED_{75} concentrations determined previously on transmurally stimulated preparations, suppressed significantly the contractions to acetylcholine at 1 and 10 ng/ml bath concentration. Clonidine and B-HT 920 had no effect on contraction of the ileum elicited by exogenous acetylcholine when tested at their ED_{50} concentrations.

hibition by clonidine on transmurally stimulated guinea pig ileum, and demonstrates a similar action of B-HT 920. Urapidil, a proven α_1-adrenoceptor antagonist (1, 2), is ineffective in inhibiting these contractions unless high concentrations are employed. The effects of clonidine and B-HT 920 are antagonized by yohimbine whereas urapidil's effect is not. The inhibitory effect due to high concentrations of urapidil is attributable to an anticholinergic mechanism because urapidil depressed contractions induced in the ileal muscle by acetylcholine, but not $PGF_{1\alpha}$. Cirazoline, which is an α_1 agonist and α_2 antagonist, also exhibits anticholinergic properties at relatively high concentrations (13). Therefore, urapidil inhibits contractions to transmural stimulation by depressing the response at a postjunctional cholinergic site; stimulation of prejunctional α_2 receptors does not appear to be involved.

Because urapidil is an effective long-lasting hypotensive agent in both conscious normotensive and Goldblatt hypertensive dogs (2) and in anesthetized animals (3), its mechanism of action is of interest. Peripheral α-adrenoceptor antagonism at both α_1- and α_2-adrenoceptor subtypes has been reported based on *in vivo* (2) and *in vitro* experiments (1) which accounts in part for the reduction in arterial blood pressure. However, a central sympathoinhibitory action of urapidil has also been demonstrated (3) which has not yet been specifically characterized. Since preganglionic sympathetic nerve activity is depressed by clonidine (14) and also by urapidil (3), Eltze (1) examined urapidil for clonidine-like activity in the intramurally stimulated rat vas deferens. Clonidine and xylazine maximally inhibited the twitch response of the vas deferens by 89 and 72%, in concentrations of 5×10^{-8} and 1×10^{-6} *M*, respectively, and were considered to act as full α_2 agonists, whereas urapidil at 1×10^{-6} *M* only inhibited the response by 52% which was maximum for this agent. He ascribed the depression of the twitch response to a partial α_2-agonist action of urapidil. We found no evidence for a clonidine-like effect of urapidil in the transmurally stimulated guinea pig ileum. We are uncertain as to why our results disagree with those found in the rat vas deferens, but we have entertained two possibilities. It was assumed by Eltze (1) that inhibition of the twitch response of the vas deferens by urapidil and antagonism of the depression by α_2 antagonists meant that urapidil exerted a prejunctional inhibitory action mediated by α_2-adrenoceptors. However, since urapidil has a potent α_1-antagonistic effect, it may depress these contractions by blocking at a postjunctional α-receptor site. Second, the flat concentration–response curve with a maximum depression of the response of only 52% suggests a relatively weak nonspecific effect of urapidil. The guinea pig ileum serves as a better model than the vas deferens for studying the prejunctional action of α-adrenoceptor antagonists since its contractions are not adrenergically mediated.

In summary, urapidil does not appear to possess a prejunctional α_2-agonistic action in guinea pig ileum. At high concentrations, urapidil inhibits the contraction to transmural stimulation by depressing the response at a postjunctional cholinergic site.

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