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INTERACTION OF PHENOXYBENZAMINE WITH MUSCARINIC RECEPTORS AND CALCIUM CHANNELS

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Abstract—Phenoxybenzamine (POB, 10^{-6}–10^{-4} M) inhibited the responses of guinea pig ileal longitudinal smooth muscle to both muscarinic agonists and K^+-depolarization but was more effective against the agonist-induced responses. POB inhibited binding of both the muscarinic antagonist [^{3}H]-quinuclidinyl benzilate (QNB) and the Ca^{2+} channel antagonist [^{3}H]nitrendipine and was, paralleling its effects on mechanical responses, more effective against [^{3}H]QNB binding. POB reduced specific [^{3}H]QNB binding by a reduction in B_{max} with no change in K_D, but inhibited [^{3}H]nitrendipine binding by reducing K_D with no effect on B_{max}. It is suggested that the activity of POB against Ca^{2+} channels may underlie the ability of POB, and other 2-halogenoethylamines, to inhibit a wide variety of apparently discrete pharmacological events.

Phenoxybenzamine (POB) was originally introduced as an irreversible α-adrenoceptor antagonist [1, 2]. Recent studies with [^{3}H]POB indicate specific irreversible binding with the properties anticipated for α-adrenoceptor labeling [3–5]. Although POB is a potent α-adrenoceptor antagonist, it and related 2-halogenoethylamines, including dibenamine, exhibit a broad spectrum of pharmacologic effects [6]. Thus, they inhibit responses elicited at acetylcholine, histamine, 5-hydroxytryptamine, dopamine and opiate receptors [2, 6–10]. Additionally, these 2-halogenoethylamines inhibit smooth muscle responses elicited by such nonreceptor stimulants as K^+-depolarization and Ca^{2+} [11–14]. This diverse range of pharmacologic effects may relate to the ability of POB, and other 2-halogenoethylamines, to interact at a variety of discrete receptor systems, but it is possible that interaction at some effector common to or shared by a variety of receptors and activation processes underlies the multiple actions of POB.

The Ca^{2+} channel is a plausible candidate for such a common effector system since its activation could be a common component of a variety of stimuli. It was suggested several years ago that POB antagonism of α-adrenoceptor function might involve both receptor and Ca^{2+} processes [15], and a similar view has been expressed more recently by El-Fakahany and Richelson [16] for muscarinic receptors in neuroblastoma cells.

The recent introduction of [^{3}H]nitrendipine makes possible more direct measurement of Ca^{2+} channels [17–19], and we have therefore compared directly the ability of POB to inhibit mechanical response, [^{3}H]nitrendipine binding and [^{3}H]quinuclidinyl benzilate binding in guinea pig ileal longitudinal muscle, a system in which Ca^{2+} channel activation [20] and muscarinic receptor, and Ca^{2+} channel, binding have been described [18, 21].

MATERIALS AND METHODS

Longitudinal muscle strips were prepared from guinea pig ileum [20] from 350–500 g male guinea pigs (Buckberg, NY). Muscle strips were set up to record isotonic contractions and both cumulative dose–response curves and single responses to the muscarinic agonists *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (CD) and methylfurmethide (MF) and to K^+ as previously described [20, 21]. Muscle strips were incubated in a saline solution of the following composition (mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaHCO_3$, 11.9; NaH_2PO_4, 0.41; and dextrose, 5.55; aerated (O_2:CO_2, 95:5), and maintained at 37°. Responses to K^+ were determined with iso-osmotic replacement of NaCl by KCl. To determine the effects of POB on mechanical response, tissues were incubated with various concentrations of POB, diluted from a freshly prepared cold 10^{-3} M stock solution, and dose–response curves or single responses to CD, MF or K^+ were determined and compared to control responses. Tissues were exposed to POB for 15 min (dose–response curves) or 60 min (single responses) and were then washed repeatedly for 60 min. Tissues were not exposed to more than one concentration of POB.

To determine the effect of POB on (−)-[^{3}H]-quinuclidinyl benzilate ([^{3}H]QNB) and [^{3}H]nitrendipine binding, slight modifications of our previously published protocols [18, 21] were employed. A microsomal preparation from guinea pig ileal longitudinal muscle was suspended in 50 mM Tris buffer (pH 7.2, 25°) at a concentration of 50–100 μg protein/

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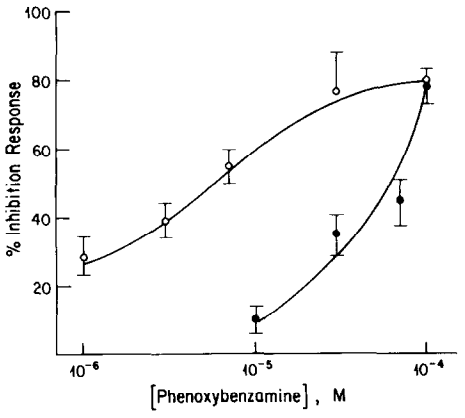

Fig. 1. Inhibitory effect of phenoxybenzamine on the mechanical responses of guinea pig ileal longitudinal smooth muscle to supramaximal concentrations of methylfurmethide (MF; 10^{-6} M) and K^+ (80 mM). Key: (○) MF; and (●) K^+: Tissues were exposed to various concentrations of POB for 60 min. Data are means ± S.E.M. (N = 4–6).

5 ml assay volume. Protein was determined by the method of Bradford [22] using bovine serum albumin as the standard. Tubes were incubated with POB for 20 min at 25° and centrifuged at 45,000 *g* for 30 min. The pellet was suspended in 10 ml of cold Tris buffer and recentrifuged at 45,000 *g* for 30 min, then resuspended and incubated with [^{3}H]QNB or [^{3}H]nitrendipine for 60 min. Specific binding was defined in a parallel set of tubes containing 10^{-7} M unlabeled nitrendipine or 10^{-6} M unlabeled atropine. A set of tubes from the same tissue preparation was treated identically but was not incubated with POB. Binding was terminated by rapid filtration through Whatman GF/B filters with two 5-ml rinses of cold buffer. The filters were counted by liquid scintillation spectrometry.

[^{3}H]QNB (New England Nuclear Corp., Boston, MA) had a specific activity of 40.2 Ci/mmole and [^{3}H]nitrendipine (New England Nuclear) a specific activity of 85 Ci/mmole. Phenoxybenzamine was a gift from Smith, Kline & French, Philadelphia, PA. *cis*-2-Methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide and methylfurmethide were synthesized in our laboratory [23, 24].

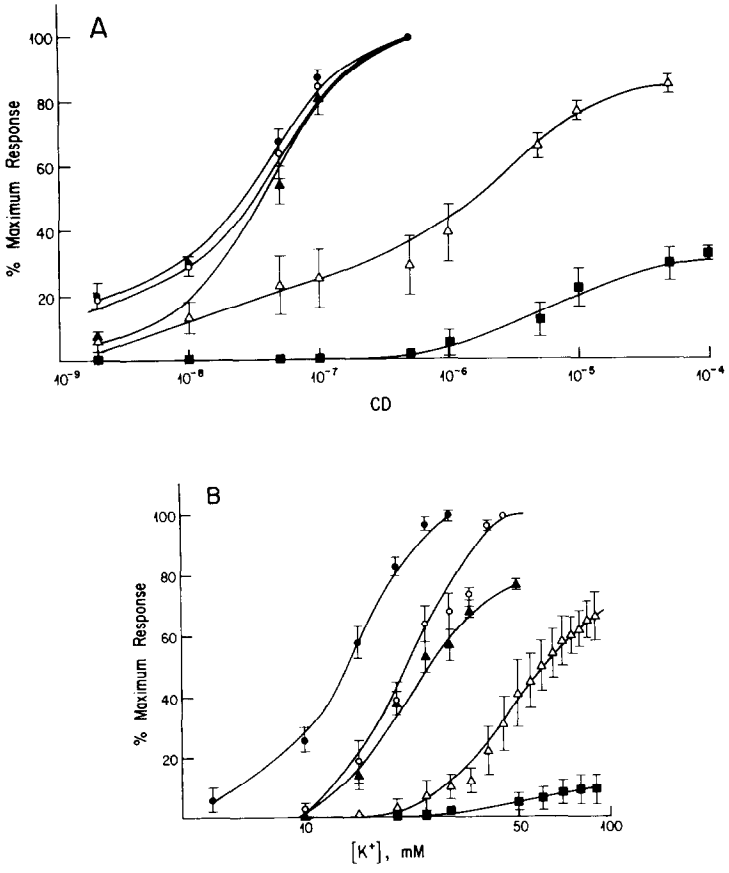

Fig. 2. Effect of phenoxybenzamine on cumulative dose–response curves to *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (CD) and K^+ in guinea pig ileal longitudinal smooth muscle. Tissues were exposed to various concentrations of POB for 15 min. (A) Responses to CD. Key: (●) control; (○) 10^{-8} M POB; (▲) 10^{-7} M POB; (△) 10^{-6} M POB; and (■) 10^{-5} M POB. (B) Responses to K^+. Key: (●) control; (○) 10^{-6} M POB; (▲) 5×10^{-6} M POB; (△) 10^{-5} M POB; and (■) 5×10^{-5} M POB. Data are means ± S.E.M. (N = 5).

RESULTS AND DISCUSSION

The mechanical responses of guinea pig ileal longitudinal smooth muscle to the muscarinic agonists CD and MF and to K^+ depolarization were progressively inhibited by increasing concentrations of POB (Figs. 1 and 2). Responses to muscarinic agonists, measured as single responses (Fig. 1) or cumulative dose–response curves (Fig. 2), were more sensitive to POB than were the corresponding K^+ depolarization-induced responses. IC_{50} values for POB inhibition of single responses to MF or K^+ were 5.6×10^{-6} M and 5.3×10^{-5} M respectively. These data confirm previous reports that POB causes irreversible inhibition of both muscarinic agonist and K^+-induced responses in smooth muscle [7, 11–14].

It is generally argued that POB inhibits muscarinic responses by progressive irreversible binding to the receptors, the initial rightward shift of the dose–response curve representing a receptor reserve [6, 23]. Radioligand binding data support this conclusion since POB causes a concentration-dependent decrease of specific [^{3}H]QNB binding (Fig. 3) with an IC_{50} of 3.9×10^{-7} M. Scatchard analysis of the effect of POB on [^{3}H]QNB binding showed a reduction in B_{max} of [^{3}H]QNB binding with no significant change in the K_D value (Fig. 4).

However, interaction at muscarinic receptors is not the only site of POB interaction in the ileal longitudinal smooth muscle. Specific binding of [^{3}H]-nitrendipine was also irreversibly inhibited by POB (Fig. 3) with an IC_{50} value of 5.1×10^{-6} M. This confirms that the ability of POB (and presumably other 2-halogenoethylamines) to inhibit K^+-induced responses in smooth muscle is, as previously suggested [15, 16], due to interaction at Ca^{2+} channels. The lower sensitivity of [^{3}H]nitrendipine binding to POB accords with the lower sensitivity of K^+-induced responses relative to muscarinic agonist-induced responses in this preparation (Figs. 1 and 2). Scat-

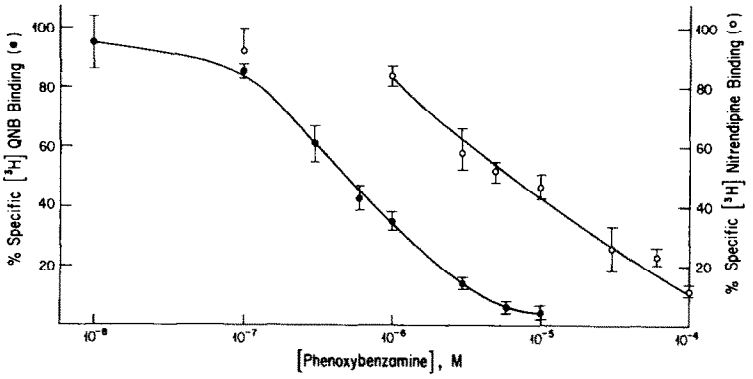

Fig. 3. Inhibitory effects of phenoxybenzamine on the specific binding of [^{3}H]quinuclidinyl benzilate (●) and [^{3}H]nitrendipine (○) to a microsomal fraction from guinea pig ileal longitudinal smooth muscle. The data are the means of three experiments each performed in duplicate. Bars indicate S.E.M.

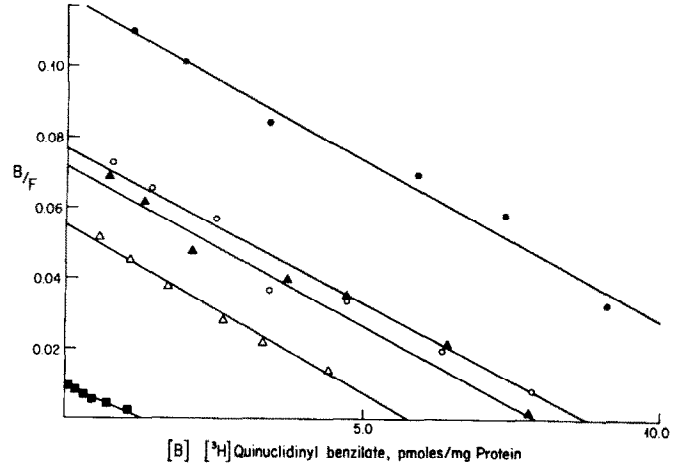

Fig. 4. Scatchard analysis of the effects of phenoxybenzamine on specific binding of [^{3}H]quinuclidinyl benzilate to a guinea pig ileal microsomal preparation. Key: (●) control, B_{max} 13.1 pmoles binding site/mg protein, $K_D = 1.1 \times 10^{-10}$ M; (○) 10^{-8} M POB, $B_{max} = 8.7$ pmoles/mg protein, $K_D = 1.54 \times 10^{-10}$ M; (▲) 10^{-7} M POB, $B_{max} = 7.8$ pmoles/mg protein, $K_D = 1.0 \times 10^{-10}$ M; (△) 10^{-6} M POB, $B_{max} = 5.7$ pmoles/mg protein, $K_D = 1.0 \times 10^{-10}$ M; and (■) 10^{-5} M POB, $B_{max} = 1.4$ pmoles/mg protein, $K_D = 1.8 \times 10^{-10}$ M. Each point is the mean of data from four experiments each performed in duplicate.

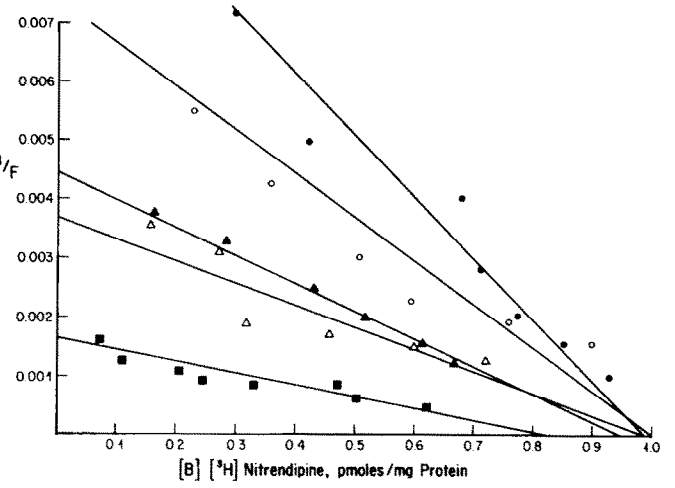

Fig. 5. Scatchard analysis of the effects of POB on specific [^{3}H]nitrendipine binding to a guinea pig ileal microsomal preparation. Key: (●) control, B_{max}, 0.98 pmole/mg protein, $K_D = 1.0 \times 10^{-10}$ M; (○) 10^{-6} M POB, $B_{max} = 1.0$ pmoles/mg protein, $K_D = 1.6 \times 10^{-10}$ M; (▲) 5×10^{-6} M POB, $B_{max} = 0.94$ pmoles/mg protein, $K_D = 2.0 \times 10^{-10}$ M*; (△) 10^{-5} M POB, $B_{max} = 0.99$ pmole/mg protein, $K_D = 2.4 \times 10^{-10}$ M*; (■) 5×10^{-5} M POB, $B_{max} = 0.81$ pmoles/mg protein*, $K_D = 4.8 \times 10^{-10}$ M*. Each point is the mean of data from four experiments each performed in duplicate. An asterisk (*) indicates significantly different from control, $P < 0.05$.

chard analysis of the POB–[^{3}H]nitrendipine interaction (Fig. 5) shows that POB reduced the K_D for [^{3}H]nitrendipine binding with a significant effect on B_{max} only at the highest POB concentration studied (10^{-4} M). These data suggest that POB is an allosteric inhibitor of [^{3}H]nitrendipine binding, a mechanism that may underlie the rightward shift of the K^+ dose–response curve produced by POB. Several other allosteric inhibitors of [^{3}H]nitrendipine binding have been reported including the Ca^{2+} channel antagonists verapamil, D-600 and diltiazem [19, 25, 26].

There are several interesting implications to the data reported here. The establishment of POB as a Ca^{2+} channel antagonist, as determined by inhibition of [^{3}H]nitrendipine binding, suggests that this may represent the common basis by which POB inhibits a number of apparently discrete pharmacologic events. Additionally, although POB is a more potent inhibitor of muscarinic than of K^+-induced events, there is, in both pharmacologic and binding experiments, incomplete separation between the two processes. Thus, the antagonism of muscarinic responses by POB is due both to inhibition of muscarinic receptor binding and to interaction at the Ca^{2+} channel. It is likely that similar interactions at both receptor sites and Ca^{2+} channels may exist in other systems where relatively high concentrations of phenoxybenzamine are used to inactivate receptors. Finally, it is interesting to speculate that the reported antiarrhythmic activity of POB [27] may be due to its ability to interact at Ca^{2+} channels.

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