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ACTIONS OF Ca^{2+} ANTAGONISTS ON THE GUINEA-PIG ILEAL MYENTERIC PLEXUS PREPARATION

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Abstract—The guinea-pig ileal myenteric plexus preparation has been used to compare the actions of three Ca^{2+} channel antagonists, D 600 (methoxyverapamil), nicardipine and diltiazem, on smooth muscle contractions and acetylcholine release in response to electrical stimulation. Acetylcholine release was not affected by these agents at concentrations of 10^{-6}–10^{-5} M. In confirmation of previous work, however, the smooth muscle contractile responses were effectively inhibited at these concentrations. Quercetin, a non-Ca^{2+} channel antagonist, was approximately equieffective in blocking both acetylcholine release and smooth muscle contractions. These data suggest that differences may exist in the antagonist sensitivity of voltage-dependent Ca^{2+} channels.

The organic Ca^{2+} antagonists including verapamil, nifedipine and diltiazem have achieved considerable pharmacologic and therapeutic importance because of their potent and quite selective abilities to block voltage-dependent Ca^{2+} currents in cardiac and smooth muscle [1–4]. Major questions remain to be resolved, however, concerning their sites and mechanisms of action [5, 6]. Of particular interest is the question of selectivity of action of these antagonists. There is evidence to indicate that not all voltage-dependent, Ca^{2+} channel mediated processes are equally sensitive to the organic Ca^{2+} antagonists [for reviews, see Refs. 5 and 7]. This appears to be the case for a number of stimulus–secretion coupling processes, notably those of nerve terminals [8–10].

To further investigate this, we have compared the actions of several organic Ca^{2+} antagonists including D 600 (methoxyverapamil), diltiazem and nicardipine (YC-93, a potent nifedipine analog [11]) in the field-stimulated guinea-pig ileal myenteric plexus preparation [12], against both contractions and acetylcholine release. Quercetin, a flavonoid compound, was also included. This compound is known to inhibit some Ca^{2+}-dependent processes, including histamine release from basophils, but not to inhibit smooth muscle contractions [13].

MATERIALS AND METHODS

Male albino guinea pigs (Buckberg) weighing 300–500 g were decapitated; the small intestines were removed with about 20 cm of terminal ileum being discarded. The intestine was cut into segments 3–4 cm in length, and the longitudinal muscle, with the myenteric plexus attached, was gently separated from the underlying circular muscle [14]. The dissected muscle strips were weighed, then attached via silk threads to a retaining rod at one end and a transducer at the other end, and mounted in 10-ml plastic organ baths containing Krebs-bicarbonate solution (118 mM NaCl; 4.7 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM $MgCl_2$; 1.2 mM NaH_2PO_4; 25 mM $NaHCO_3$; and 11 mM glucose) with choline chloride (0.03 mM) at 37° and bubbled with 95% O_2/5% CO_2 to maintain the pH at 7.4. The myenteric plexus-longitudinal muscle was suspended under a resting tension of 0.3 g and allowed to equilibrate for 45 min with several changes of solution. After equilibration, the tissue was conditioned by adding 10 μM acetylcholine to the bath. Isometric contractions of the longitudinal muscle, that were produced by acetylcholine, were recorded by a Narco Myograph "A" force transducer coupled to a Narco Physiograph CPM polygraph recorder. After 1–2 min, the drug was washed from the bath with four rapid rinses with Krebs-bicarbonate solution. The preparation was allowed to recover for an additional 12 min.

The effects of Ca^{2+} antagonists on the reactivity of the myenteric plexus-longitudinal muscle to electrical field stimulation [12] and on the basal and evoked release of acetylcholine from the myenteric plexus were examined according to a modification of the method of Down and Szerb [15]. Each tissue was stimulated via two platinum wire ring electrodes 4 cm apart, positioned at the top and bottom of the organ bath. Repeated supramaximal rectangular pulses (*ca.* 40 V) of 1.5 msec duration at a frequency of 0.1 Hz were generated by a Grass S88 stimulator. Each pulse elicited a single contraction. Tissues were supraximally stimulated for 90 min to obtain a stable baseline of isometric contractions. These contractions were defined as control responses for each preparation. During the final 15 min of stimulation, 10 μCi [^{3}H]choline ([^{3}H]methylcholine chloride, 80.0 Ci/mmole, New England Nuclear Corp., Boston, MA) was added to the bathing solution. After discontinuing stimulation, the tissues were washed eight times, once every 10 min, with Krebs-bicarbonate solution containing 50 μM hemicholinium-3.

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Triplicate 0.05-ml aliquots of each bathing solution were collected 10 min after the last wash and, again, from each preparation at the end of two further 10-min periods. These samples were used to measure the basal efflux. D 600, diltiazem, YC-93 or quercetin was then added to the test preparations. A control preparation was run simultaneously with each set of treated preparations. All preparations were then supramaximally stimulated for 10 min, and a final set of triplicate aliquots was collected. Aliquots were transferred to scintillation counting vials containing 5 ml PCS (Amersham, Arlington Heights, IL), and the radioactivity was determined by liquid scintillation spectrometry at a counting efficiency of 35%. The amount of radioactivity released during each period was determined by subtracting the counts collected in the previous period. Results were expressed as pmoles [^{3}H]acetylcholine released per g tissue wet weight per min. The natures of the ^{3}H-material released during rest and during stimulation probably differed. During rest a mixture of choline, phosphorylcholine and acetylcholine is released, but low frequency stimulation increases only the release of acetylcholine [15]. Contraction amplitudes were measured at the end of the stimulation period and are reported as percentages of control.

Drug solutions were prepared before the experiment by dissolving the agents in distilled water, except YC-93 and quercetin, which were dissolved in ethanol. Final bath concentrations were obtained by adding 10–30 μl volumes of stock drug solution to the organ baths. Separate control experiments showed that this volume of ethanol was without effect on tissue responses. YC-93 was a gift from the Yamanouchi Pharmaceutical Co., Tokyo, Japan; diltiazem a gift from Marian Laboratories, Kansas City, MO; and D 600 a gift from Knoll A.G., Ludwigshaffen, FRG. Quercetin was purchased from the Aldrich Chemical Co, Milwaukee, WI.

Data were analyzed by Student's t-test. The level of statistical significance chosen was $P < 0.05$.

RESULTS

The organic Ca^{2+} channel antagonists D 600, diltiazem and YC-93, when employed at 10^{-6} and 10^{-5} M, inhibited the twitch response to field stimulation of the myenteric plexus-longitudinal muscle. Figure 1 demonstrates this for the 10^{-5} M concentration of these agents; inhibition at 10^{-6} M (not shown) was essentially identical.

At these same concentrations, D 600, diltiazem and YC-93 were without effect on stimulated release of acetylcholine (Fig. 2).

In marked contrast, quercetin (10^{-6}–10^{-4} M) was approximately equiactive against twitch responses (Fig. 3) and stimulated acetylcholine release (Fig. 2).

DISCUSSION

The responses of the guinea-pig ileal longitudinal smooth muscle to acetylcholine or K^+ are known to be highly dependent upon extracellular Ca^{2+} and to be very sensitive to the inorganic antagonists (Mn^{2+}, La^{3+}) and to the organic Ca^{2+} channel antagonists [16, 17]. Field-stimulated acetylcholine release in this preparation is, as are other neurotransmitter release processes, dependent upon extracellular Ca^{2+} and inhibited by Mn^{2+} [18, 19]. Thus, a useful comparison of Ca^{2+} antagonist action at neuronal and smooth muscle Ca^{2+} channels is possible in this preparation.

The organic Ca^{2+} channel antagonists D 600, diltiazem and YC-93 inhibited the contractile response

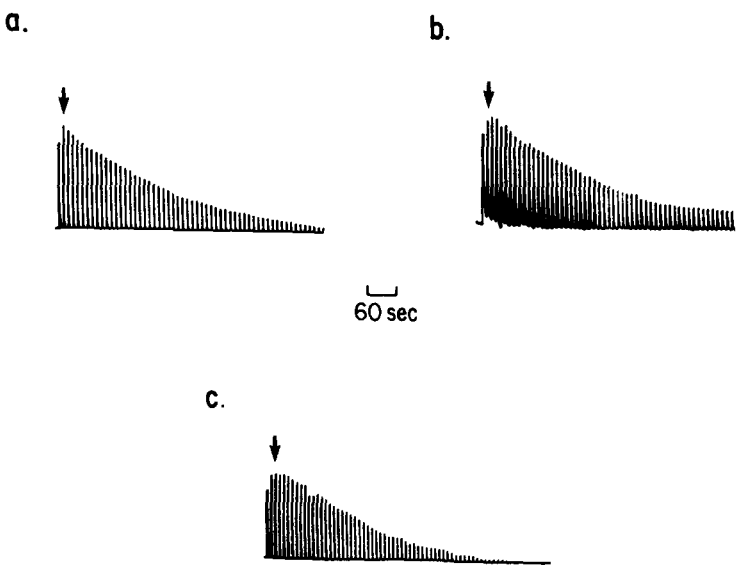

Fig. 1. Inhibition by (a) D 600 (10 μM), (b) diltiazem (10 μM), and (c) nicardipine (10 μM) of the twitch response to field stimulation of the myenteric plexus-longitudinal muscle of the guinea-pig ileum. The tissues were suspended at a tension of 300 mg in a 10-ml organ bath containing Krebs-bicarbonate solution at 37°. Twitch responses were elicited by repeated supramaximal rectangular pulses (1.5 msec at 0.1 Hz).

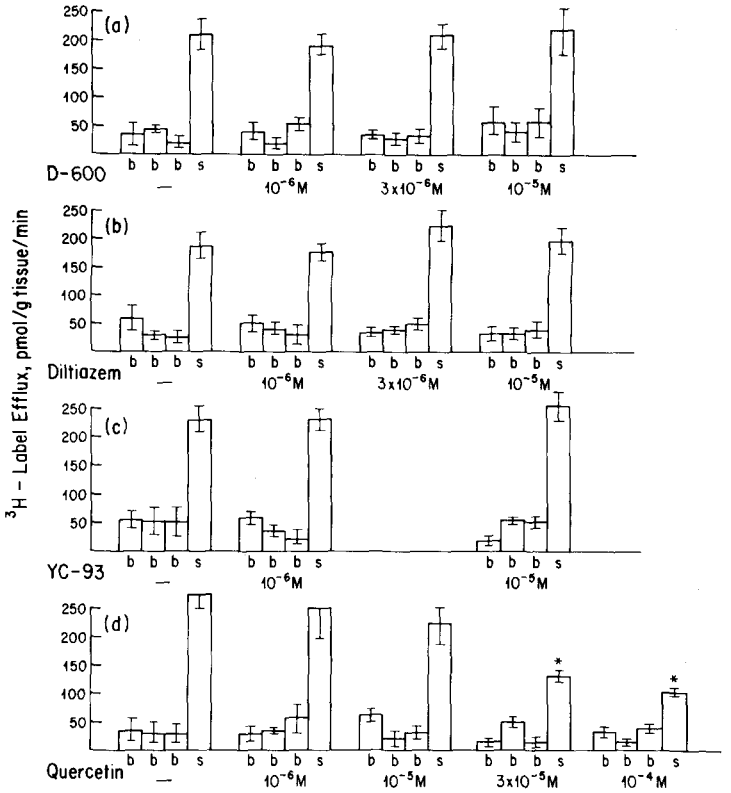

Fig. 2. Effects of (a) D 600 (10^{-6}–10^{-5} M), (b) diltiazem (10^{-6}–10^{-5} M), (c) YC-93 (10^{-6}–10^{-5} M) and (d) quercetin (10^{-6}–10^{-4} M) on stimulus-evoked ^{3}H-label release from guinea-pig ileal longitudinal muscle preloaded with [^{3}H]choline. Basal release (b) was measured for three consecutive 10-min periods, and the effect of the antagonist was measured during a subsequent 10-min supramaximal stimulation period (s). A control set of tissues was treated identically save that antagonist was not added. The vertical bars in each column represent S.E.M. for four preparations. An asterisk (*) indicates significant difference ($P < 0.05$) from control. The increase in release of ^{3}H-label during stimulation over basal release is attributable to [^{3}H]acetylcholine release.

evoked by acetylcholine release in the electrically stimulated guinea-pig ileal myenteric plexus-longitudinal muscle preparation. The virtually complete abolition of twitch responses by these agents at concentrations of 10^{-5} M (and 10^{-6} M) accords with previous observations in this tissue of the high sensitivity of acetylcholine responses to the Ca^{2+} channel antagonists [16, 17]. However, the process of acetylcholine release was quite insensitive to the same concentrations of these antagonists.

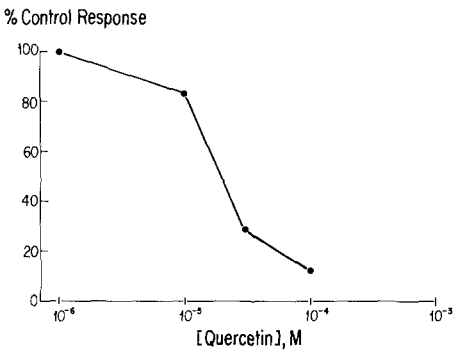

Fig. 3. Concentration–response curve for the relaxation elicited by quercetin in the field-stimulated myenteric plexus-longitudinal muscle of the guinea-pig ileum. Each point represents the mean of two to four experiments. The S.E.M. (not shown) did not exceed ± 10%.

In contrast, quercetin inhibited with approximately equal effectiveness both twitch responses and evoked acetylcholine release. Quercetin is not a Ca^{2+}-channel antagonist, and its inhibitory actions against Ca^{2+}-dependent processes, including mast cell histamine release [20], have been attributed to interference with membrane Ca^{2+}-ATPase [21–23] or to inhibition of phosphodiesterase [24].

The insensitivity of Ca^{2+} currents in neuronal systems to the organic Ca^{2+} channel antagonists has been documented previously in adrenergic nerve terminals [8], the frog neuromuscular junction [9, 10] and rat brain synaptosomes [10]. Atlas and Adler [25] have shown that the Ca^{2+} currents in neuroblastoma-glioma hybrid cells are also very much less sensitive to D 600 inhibition than are the currents in cardiac and smooth muscle cells. Clearly, the Ca^{2+} channels in myenteric neurons also fall into this organic Ca^{2+} antagonist-insensitive class. This insensitivity is presumably a function of the structure and/or kinetics of the Ca^{2+} channel, since it is shared by D 600, diltiazem and YC-93, each representing a different class of antagonist.

Thus, although inward Ca^{2+} currents are required for the stimulus-secretion coupling process of neurotransmitter release [26–29] and for many excitation–contraction coupling processes in smooth and cardiac muscle [1, 2, 5, 7], the Ca^{2+} channels that mediate each appear to be differentially sensitive to the organic Ca^{2+} channel antagonists.

Collectively, these data may indicate the existence of distinct subclasses of Ca^{2+} channels [30, 31]. Clearly, further work is needed to justify any such speculation, but there is precedent both with pharmacological receptors [32] and Na^{+} channels [33].

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