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Augmentation of the Neural Inhibitory Response of the Lower Esophageal Sphincter (37942)

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It has been shown recently that the circular smooth muscle of the physiological lower esophageal sphincter (LES) contains nonadrenergic inhibitory nerves (1). It has been suggested that the neurotransmitter through which the nonadrenergic neural inhibitory system functions is adenosine triphosphate or a related purine nucleotide (2, 3). Although no specific antagonist is available to determine selectively the neurotransmitter involved in nonadrenergic neural inhibition, it has been suggested that this neural response could be potentiated selectively by compounds acting to block the intracellular uptake of adenosine (3–5). The purpose of this study was to determine the effect of the adenosine-uptake inhibitors dipyridamole and lidoflazine on the neural inhibitory response in LES circular muscle.

Methods. All studies were performed in the opossum (*Didelphis virginiana*) using methods previously described in detail (1). The physiological LES was identified in each animal, in vivo, using perfused open-tipped catheters to detect the high-pressure zone. The animals were killed and the muscle strips from the LES were obtained. Each muscle strip, 0.5 cm in width and 1.0 cm in length, was mounted to record tension from the plane of contraction of the circular muscle. Each muscle was studied during the superfusion of Krebs–Ringer solution bubbled with 95% O_2 and 5% CO_2 maintained at 36°–38° as described previously (1). Acetylcholine (10^{-4} *M*) and physostigmine (4.0×10^{-7} *M*) were utilized to establish sustained plateaus of muscle contraction.

Force transducers (Grass Ft. 03C) measured isometric tension which was graphed on a direct-writing rectilinear Beckman recorder. Each muscle was set at its length of optimal tension development, Lo, for the duration of each study. Each muscle was stimulated electrically by two platinum wires adjusted to juxtapose the lateral longitudinal muscle strip surfaces. A square-wave stimulator (Grass model S44, with stimulus isolation unit SIU 5) delivered 30-sec trains at 1 msec duration, direct-current, square wave pulses at 10 Hz. Voltage was varied from 10 to 100 V.

Each muscle was used as its own control. The percent inhibition of the active tension achieved with the superfusion of acetylcholine or 60 m*M* KCl was quantified during electrical stimulation at 10–100 V. Dipyridamole (Persantine, Geigy Pharmaceuticals, Ardsley, NY), lidoflazine (Clinium, McNeil Laboratories, Fort Washington, Pa), or theophylline was added to the superfusate and the inhibitory response was again recorded at each stimulus parameter.

Results. In Fig. 1 are shown the stimulus response characteristics of LES circular muscle contracted with a superfusion of acetylcholine (10^{-4} *M*) and physostigmine (4.0×10^{-7} *M*). Responses were compared for the control and for dipyridamole (5.0×10^{-7} g/ml), lidoflazine (10^{-7} g/ml), and theophylline (5.0×10^{-5} g/ml). In the presence of dipyridamole, the inhibitory response of the muscle was increased significantly at 30 V ($P < 0.05$) and 60 through 100 V ($P < 0.01$). Lidoflazine potentiated the inhibitory responses at 30,

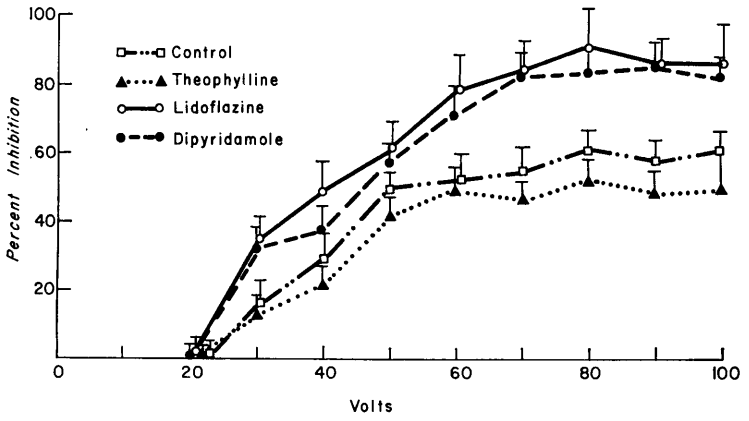

FIG. 1. The stimulus response characteristics of LES circular smooth muscle during the superfusion of dipyridamole (5.0×10^{-7} g/ml), theophylline (5.0×10^{-5} g/ml), and lidoflazine (10^{-7} g/ml). The percent inhibition of acetylcholine ($10^{-4} M$) and physostigmine ($4.0 \times 10^{-7} M$) contracted muscle is plotted as a function of increasing voltage. Each point represents the mean plus 1 SEM for studies obtained on muscle strips from a minimum of eight animals. Dipyridamole and lidoflazine significantly potentiated inhibition. Theophylline had no significant effect at any voltage.

40, and 50 V ($P < 0.05$) as well as 60 through 100 V ($P < 0.01$). Theophylline did not significantly alter the inhibitory response at any voltage ($P > 0.05$). Neither dipyridamole nor lidoflazine altered the threshold level at which inhibition was obtained.

In Fig. 2 are shown the stimulus response characteristics of LES circular muscle contracted with a superfusion of 60 mM KCl. The control response was compared to the response achieved in the presence of dipyridamole (5.0×10^{-7} g/ml) and lidoflazine (10^{-7} g/ml). Again, both dipyridamole and lidoflazine augmented the inhibition of LES muscle. Significant increases in the degree of inhibition were seen at 40, 50, 70, 80, and 90 V for dipyridamole and at 40 through 80 V for lidoflazine ($P < 0.05$). The magnitude of inhibition of the LES

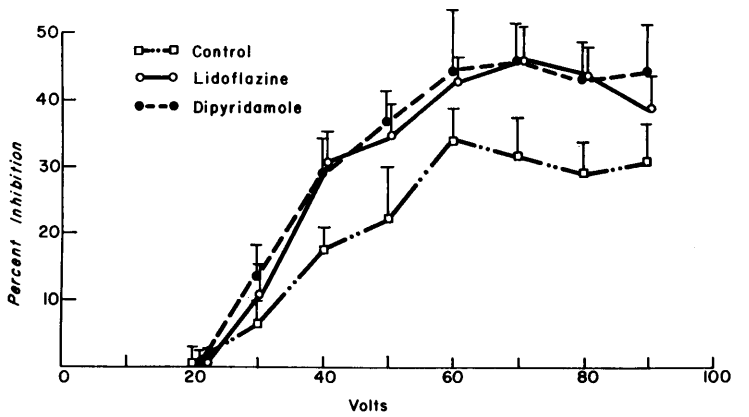

FIG. 2. The stimulus response characteristic of LES circular smooth muscle during the superfusion of dipyridamole (5.0×10^{-7} g/ml) and lidoflazine (10^{-7} g/ml). The muscle was contracted in response to a 60 mM KCl depolarization. Lidoflazine and dipyridamole potentiated the inhibitory response.

muscle during KCl depolarization was less than noted during acetylcholine superfusion.

Discussion. The purpose of this study was to determine whether compounds that inhibited the intracellular uptake of adenosine altered the inhibitory response of LES circular muscle during electrical stimulation. The results of these studies indicated that both dipyridamole and lidoflazine significantly potentiated the electrically induced inhibition of LES muscle during either an acetylcholine-stimulated contraction or KCl depolarization.

The presence of nonadrenergic inhibitory nerves in the gastrointestinal tract has been recognized for many years (6). Recently, these nerves have been proposed as the mechanism of LES relaxation during swallowing (1). Although one can readily demonstrate that inhibition in LES muscle is a nonadrenergic, neural response, it is difficult to selectively establish the neurotransmitter mediating this response. There are no compounds which selectively antagonize the neurotransmitter in question. However, it has been proposed recently that nonadrenergic inhibitory nerves release adenosine triphosphate or a related purine nucleotide (2, 3).

The suggestion that the neurotransmitter was adenosine triphosphate has been supported by the demonstration that compounds acting as blockers of the intracellular uptake of adenosine potentiated inhibition in muscle containing nonadrenergic inhibitory nerves but not adrenergic nerves (3, 4). Additionally, blockers of adenosine uptake potentiated inhibition of muscle in response to the direct application of adenosine triphosphate (4). In the studies presented here, both dipyridamole and lidoflazine potentiated the inhibitory response of the LES muscle to electrical stimulation. This observation supports the concept that the nonadrenergic inhibitory nerves mediating inhibition in LES muscle may indeed release adenosine triphosphate or a related purine nucleotide. However, there is no direct support for this suggestion. It is possible that the potentiating effect of both dipyridamole or lidoflazine may be through another mechanism. Dipyridamole does act as a phosphodiesterase inhibitor as well as an adenosine-uptake inhibitor (3, 7). However, a known potent phosphodiesterase inhibitor, theophylline, did not potentiate the inhibitory response in LES muscle. This observation suggested that the dipyridamole effect was not through its phosphodiesterase action. The absence of potentiation of nonadrenergic inhibition by the phosphodiesterase inhibitors, caffeine, and theophylline has been reported (2, 3).

Pharmacological potentiation of the nonadrenergic mediated inhibition of LES muscle did not establish that a purine neucleotide was the neurotransmitter of this response. However, the observations are supportive. This ability to potentiate inhibition in LES muscle may prove to be clinically relevant in disorders such as achalasia where sphincteric relaxation is impaired.

Summary. The purpose of this study was to determine the effect of lidoflazine and dipyridamole upon the nonadenergic inhibitory response of the lower esophageal sphincter (LES) circular muscle *in vitro.* Both dipyridamole and lidoflazine significantly potentiated the maximal inhibitory responses of LES muscle to electrical stimulation during muscle contraction produced by either acetylcholine or KCl depolarization. Theophylline did not alter the magnitude of the inhibitory response. These studies indicate: (1) LES muscle neural inhibition may be potentiated by compounds known to block the intracellular uptake of adenosine, and (2) nonadrenergic inhibition of LES muscle may be through adenosine triphosphate or a related purine nucleotide as proposed for other muscles responding similarly to dipyridamole and lidoflazine.

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