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MODULATION OF AUTONOMIC NEUROEFFECTOR TRANSMISSION BY NITRIC OXIDE IN GUINEA PIG ILEUM

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N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of nitric oxide synthesis, markedly enhanced tonic ("hump") responses to transmural stimulation in guinea pig ileum longitudinal muscle. The enhancement of the hump responses was probably due to a prejunctional effect on substance P-like neurotransmission, since the action of L-NMMA was exerted also in the presence of atropine, and since responses to substance P, a mimic of nerve stimulation, were unaffected by L-NMMA as were cholinergic twitch responses and the overflow of [^{3}H]choline. Further in support, the hump responses were blocked by the substance P antagonist Spantide. All effects of L-NMMA were stereospecifically reversed by L-arginine. Endogenous nitric oxide thus selectively modulates peptidergic neurotransmission in the gut.

The vascular endothelium produces a number of substances with potential neuromodulating capacity, including prostaglandins, peptides such as endothelin and the recently discovered endothelium-derived relaxing factor (EDRF), nitric oxide (NO). Prostaglandins may modulate both adrenergic and cholinergic neurotransmission, either pre- or postsynaptically (see 1). Endothelin has been found to modulate the release of both adrenergic and cholinergic neurotransmitters (2-3), and endothelium-derived products have been suggested to inhibit noradrenaline release (4-5). Since an NO-forming enzyme has been found in the CNS (6-8) and our initial studies indicated that NO modulates autonomic neuroeffector transmission (9), the aim of the present study was to characterize this modulation by use of stereospecific substrate- and inhibitor-interaction with NO formation, and by measurement of cholinergic transmitter release.

MATERIALS AND METHODS

General. Guinea pigs of either sex (300-500g) were stunned and bled. The longitudinal muscle of the small intestine, including its Auerbach plexus, was prepared and mounted in 5 ml organ baths. The tissue was kept in Tyrode's solution (composition in mM: Na 149, K 2.9, Ca 1.8, Mg 0.5, Cl 144, HCO_3 24, H_2PO_4 0.4 and glucose 5.5) maintained at 37°C and continuously aerated with 5% CO_2 in O_2. Transmural stimulation was applied by means of a Grass S44 stimulator,

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and recordings of mechanical muscle activity were made isotonically at a load of 2 mN using Harvard transducers and BBC SE-120 writers.

[^{3}H]acetylcholine release. Guinea pig ileum longitudinal muscle was preincubated for 1 h in Tyrode's solution containing 15 μCi/ml ([^{3}H]methyl)-choline (15 Ci/mmol, Amersham, UK) and 1 μM unlabelled choline, during continuous field stimulation at 0.2 Hz. After preincubation, the tissues were suffused (1 ml/min) with Tyrode's solution, containing hemicholinium-3 (10 μM). Transmural nerve stimulation (3 Hz, 0.2 ms, 60 V, 180 pulses) was applied at 10 min intervals. Stimulation-induced overflow of transmitter was estimated from the evoked fractional release of radiotracer, and expressed as % of the first stimulation (S1), as previously described (3).

Drugs: Acetylcholine chloride, hemicholinium-3, atropine sulfate, L- and D-arginine were from Sigma (St. Louis, MO, USA), and Spantide and substance P from Peninsula (LaJolla, CA, USA). L- and D-NMMA were synthetized (10), and used as acetates.

Statistics. Student's t-test for paired data was used. Values are given as means ± SEM.

RESULTS

When transmurally stimulated at 3 Hz for 60 s at 4-6 min intervals, the ileum preparations responded with a biphasic response, consisting of a brief twitch and a prolonged plateau or tonic "hump" response (Fig. 1). The twitch response is due to release of acetylcholine whereas the hump contraction has been suggested to be due to release of substance P (11). Accordingly, in our preparations atropine almost abolished the twitch and partially inhibited the hump phase (Fig. 2). The remainder of the twitch and hump phases were mimicked by substance P application (Fig. 3) and were markedly inhibited or abolished by application of the substance P antagonist Spantide, 10^{-5} - $3x10^{-5}$ M (data not shown and Fig. 2).

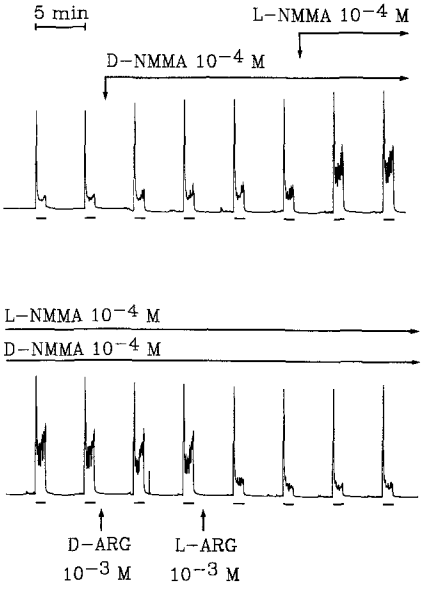

Fig. 1. Twitch and tonic ("hump") contractile responses in guinea pig ileum longitudinal muscle, evoked at 5 min intervals by transmural nerve stimulation as indicated by horizontal markers (3 Hz, 0.2 ms, 60 V, 180 pulses). There is weak enhancement by D-NMMA and marked enhancement by L-NMMA especially of the hump contractile responses, and lack of reversal by D-arginine but prompt reversal by L-arginine. The lower panel is a direct continuation of the recording in the upper panel.

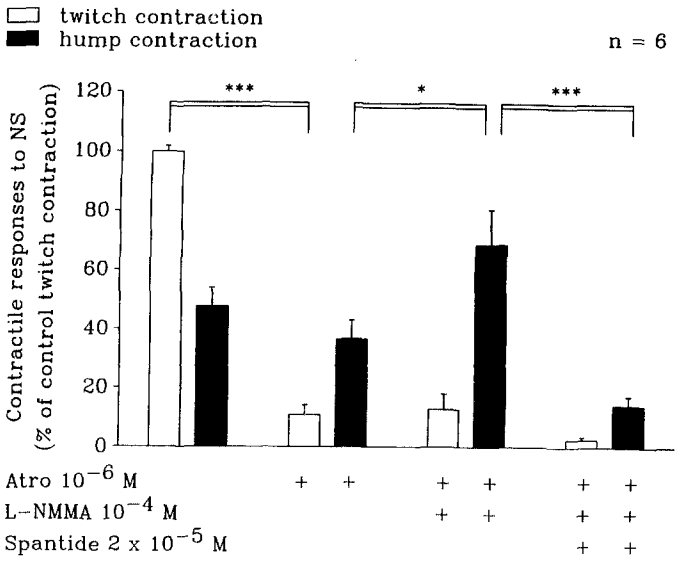

Fig. 2. Twitch and tonic ("hump") contractile responses in guinea pig ileum longitudinal muscle, evoked at 4 min intervals by transmural nerve stimulation (3 Hz, 0.2 ms, 60 V, 180 pulses). Summary of effects of L-NMMA on twitch (open bars) and hump responses (closed bars) in atropine-treated preparations. The responses were expressed as % of a twitch contraction evoked by similar transmural stimulation 12 min prior to the last untreated control stimulation (far left pair of bars). * = $p<0.05$, *** = $p<0.001$ (Student's t-test for paired data).

L-NMMA ($3x10^{-6}$ - $3x10^{-4}$ M) markedly and in a dose-dependent fashion enhanced the tonic hump responses to transmural nerve stimulation, with a maximal enhancement at 10^{-4} - $3x10^{-4}$ M. D-NMMA (Fig. 1) was more than 10 times less effective. The effect of L-NMMA was reversible upon washing (data not shown), was virtually unaffected by application of D-arginine but fully reversed by L-arginine (Fig. 1). The enhancement of the tonic hump response by L-NMMA in unpretreated preparations was parallelled by a similar enhancement of the hump response in the presence of atropine (Fig. 2). This enhanced response was still blocked by application of Spantide, 10^{-5} - $2x10^{-5}$ M (Fig. 2). In the presence of atropine, the effect of L-NMMA on hump responses was effectively reversed by L-arginine (Fig. 2).

Application of L-NMMA (10^{-4} M) failed to enhance the contractile responses to exogenous substance P, although the tonic hump responses to transmural nerve stimulation were clearly

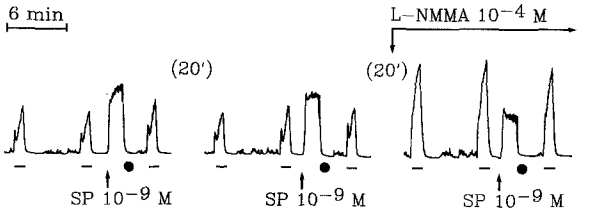

Fig. 3. Contractile responses to exogenously applied substance P in guinea pig ileum longitudinal muscle. Wash at dots. Contractile responses to transmural nerve stimulation were also evoked at 6 min intervals as indicated by horizontal markers (3 Hz, 0.2 ms, 60 V, 180 pulses). Note enhancement by L-NMMA of responses to transmural stimulation, and lack of effect on responses to substance P.

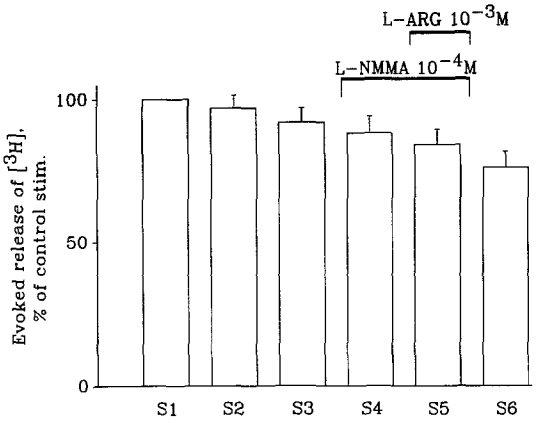

Fig. 4. Release of [^{3}H] from guinea pig ileum longitudinal muscle evoked by transmural nerve stimulation (3 Hz, 0.2 ms, 60 V, 180 pulses at 10 min intervals) of preparations preincubated with ([^{3}H]methyl)-choline. The stimulation-induced release (S2, S3, S4 etc) was measured as fractional release of radiotracer overflowing during the stimulation period and the two ensuing 2 min sampling periods, and was expressed as a percent of the first such sampling period (S1). Note the lack of effect of L-NMMA and L-arginine, applied as indicated by horizontal bars. n=5.

enhanced in the same preparations (Fig. 3). Application of L-arginine in unpretreated preparations did not modify the contractile responses to transmural nerve stimulation or contractions induced by exogenous acetylcholine or substance P (not shown). There was no significant effect of L-NMMA (10^{-4} M) on the cholinergic twitch response or on contractile responses to application of acetylcholine (not shown). Transmural stimulation evoked a reproducible overflow of [^{3}H] from preparations prelabelled with [^{3}H]choline (Fig. 4). Neither the application of L-NMMA, nor the addition of L-arginine, altered the overflow of radiotracer (Fig. 4), although contractile responses were markedly enhanced by L-NMMA in these experiments (not shown).

DISCUSSION

The present findings clearly indicate a role for endogenous NO as a modulator of neurotransmission. This is indicated by the stereospecific enhancing effect of L-NMMA on the nerve-induced hump contractions. L-NMMA has been shown to be an inhibitor of the enzyme(s) forming NO from L-arginine, both in peripheral tissues (cf. 12) and in the nervous system (6,7,13), and the fact that the effect of L-NMMA we observed was reversed by L- but not D-arginine, suggests an effect via inhibition of NO formation.

Endogenous NO seemed to be acting by inhibiting substance P-like transmission, whereas the cholinergic transmission was not significantly affected. The site of action for endogenous NO was most probably a prejunctional inhibition of the release of a substance P-like principle, since contractile responses to exogenous substance P were unaffected, and the enhanced responses to nerve stimulation in the presence of L-NMMA were blocked by Spantide.

Previous studies (9, 14), together with the present data, thus seem to argue against the concept of EDRF-induced modulation of the release of the classical neurotransmitters noradrenaline and acetylcholine, although it has been suggested that the endothelium modulates the release of

noradrenaline (4, 5). Endothelium modulation of noradrenaline release might be explained by neuromodulation by other endothelium-derived products such as endothelin (2, 3). The present data indicate a selective modulation by NO of a peptidergic substance P-like neurotransmission. A number of important issues emerge, such as the site of formation and mechanism of action of NO, as well as the regulation of its formation. Of importance is whether peptidergic neuromodulation by NO is a general phenomenon. In favour of a more general role, L-NMMA enhances excitatory NANC transmission in the pulmonary artery (9).

In conclusion, our data suggest that endogenous NO inhibits peptidergic excitatory neurotransmission to intestinal smooth muscle in the guinea pig, and this effect is likely exerted via inhibition of neurotransmitter release.

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