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# Methysergide induces selective potentiation in cholinergic contractions of the guinea-pig vas deferens by facilitating acetylcholine release

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Methysergide ($3 \times 10^{-6}$ M) enhanced the contractile responses of the isolated stripped vas deferens of guinea-pig to acetylcholine(ACh) and arecoline, but not those to noradrenaline, tyramine and bradykinin. Methysergide ($3 \times 10^{-5}$ M) suppressed the contraction elicited by noradrenaline or histamine. The methysergide-induced potentiation of the response to ACh was prevented by pre-addition of hemicholinium but not by tetrodotoxin or morphine. The augmentation of the response to ACh by physostigmine was unaffected by hemicholinium. The phasic contraction of the tissue elicited by 30 mM KCl was also enhanced by methysergide, and this enhancement was prevented by the pre-addition of atropine ($1{\cdot}4 \times 10^{-7}$ M). In the depolarized vas deferens after exposure to 30 mM KCl, methysergide occasionally induced a sustained tonic contraction which was inhibited by atropine. These findings suggest that methysergide facilitates a release of ACh by acting on the cholinergic nerve terminals and selectively potentiates the cholinergic response.

The 5-hydroxytryptamine (5-HT) antagonist, methysergide has been reported to increase the acetylcholine (ACh)-induced contraction of the isolated human intestine (Metcalfe & Turner 1970). However a convincing explanation of the mechanisms involved in the potentiation was not made.

The guinea-pig vas deferens is well accepted as an adrenergic dominant tissue, little attention has been paid to its use for evaluating the acetylcholine-like effects of drugs. However, it has been amply demonstrated from morphological (Watanabe 1969; Furness & Iwayama 1972) and pharmacological (Birmingham 1966; Thoa & Maengwyn-Davies 1968) evidence that the cholinergic terminal axon and its postsynaptic receptor site exist in the vas deferens smooth muscle.

We have found that methysergide potentiated the contractile responses of guinea-pig vas deferens to acetylcholine-like agents and the present investigation was an attempt to understand the mechanism involved in this potentiation.

## MATERIALS AND METHODS

Male guinea-pigs, 300–500 g were killed by a blow to the back of the head. Both vasa deferentia were removed, dissected free of mesentery and vascular tissue to prepare a stripped preparation, and suspended in 10 ml organ bath containing Tyrode solution composed of (mM) NaCl 137, $CaCl_2{\cdot}2H_2O$ 1·8, KCl 2·7, $MgCl_2{\cdot}6H_2O$ 1·1, $NaHCO_3$ 11·9, $NaH_2PO_4$ 0·4, and anhydrous D-glucose 5·6, and bubbled with $O_2$.

Isometric contractions were recorded on the Grass polygraph using a force displacement transducer (Grass FT-03). A resting tension of 1 g was used. The preparation was allowed to equilibrate for 30 min before the experiment. Concentrations of acetylcholine-like agonists used corresponded to ED20–ED50 values for the contractile response of the tissue.

Statistical analysis was performed using the paired sample *t*-test.

The following drugs were used: acetylcholine chloride (ACh, Sankyo), arecoline hydrochloride (Nakarai), carbamylcholine chloride (carbachol, Sigma), noradrenaline hydrochloride (NA, Sankyo), tyramine hydrochloride, dopamine hydrochloride (Wako), bradykinin triacetate (Nakarai), histamine hydrochloride, atropine sulphate, hemicholinium-3 (HC-3, Aldrich), morphine hydrochloride, crystalline tetrodotoxin (TTX, Sankyo), physostigmine salicylate (Merk), methysergide bimaleate (Sandoz).

## RESULTS

### *Selective potentiation by methysergide of contractions induced by acetylcholine-like agents*

Methysergide at concentrations up to $10^{-4}$ M did not produce any contraction of the vas deferens. However, the contractile responses of the tissue to ACh ($10^{-6}$ M), arecoline ($2 \times 10^{-5}$ M) and carbachol ($3 \times 10^{-6}$ M) were significantly potentiated by methysergide ($5 \times 10^{-6}$ M) for 5 min, whereas those to NA

* Correspondence.

($10^{-6}$ M), tyramine ($10^{-6}$ M), dopamine ($3 \times 10^{-5}$ M), and bradykinin ($3 \times 10^{-7}$ M) were not affected (Table 1). A higher concentration of methysergide ($5 \times 10^{-5}$ M) further enhanced the contractile responses to ACh-like agents, such as ACh ($10^{-6}$ M) and arecoline ($2 \times 10^{-5}$ M), but inhibited those to NA ($10^{-6}$ M) and to histamine ($3 \times 10^{-6}$ M). (Fig. 1).

Cumulative concentration-response curves for ACh, ($10^{-7}$, $10^{-6}$, $10^{-5}$, and $10^{-4}$ M) were determined in the presence and absence of methysergide. The curve for ACh, at $10^{-6}$ and $10^{-5}$ M, was displaced significantly to the left by the addition of methysergide 5 min before the first introduction of ACh ($10^{-7}$ M) (Fig 2).

*Effects of TTX, morphine, and HC-3 on the methysergide- or physostigmine-induced potentiation in contractile response to ACh*

The potentiation by methysergide ($5 \times 10^{-5}$ M for 5 min) of the contractile response to ACh ($10^{-6}$ M) was prevented by the addition of HC-3 ($10^{-4}$ M) 20 min before methysergide administration ($P$ 0·01), but not affected by TTX ($3 \times 10^{-7}$ M) or morphine ($3{\cdot}5 \times 10^{-5}$ M) 5 min before methysergide. Conversely, the augmentation by physostigmine ($2{\cdot}3 \times 10^{-7}$ M) in response to ACh was barely altered by the pre-addition of HC-3 ($10^{-4}$ M). Physostigmine and HC-3 were added to the bath 5 min and 25 min before adding ACh, respectively (Fig. 3).

*Effect of methysergide on the $K^+$-induced contraction*

The phasic contraction of the vas deferens induced by KCl (30 mM) was markedly enhanced by pre-treatment with methysergide ($3 \times 10^{-5}$ M for 5 min). This enhancement was inhibited by treatment with atropine ($1{\cdot}4 \times 10^{-7}$ M) before methysergide (Fig. 4). In a few cases, methysergide itself produced a continuous tonic contraction in the depolarized vas deferens that had been exposed to 30 mM KCl. This contraction was immediately inhibited by atropine ($4{\cdot}3 \times 10^{-7}$ M).

Table 1. Effects of methysergide on the contractile responses of guinea-pig vas deferens to agonists

| Agonist | Dose (M) | Response ratio† to control after methysergide ($5 \times 10^{-6}$ M) |
|---|---|---|
| ACh (5) | $10^{-6}$ | 1·52 ± 0·15* |
| Arecoline (5) | $2 \times 10^{-5}$ | 1·70 ± 0·12** |
| Carbachol (5) | $3 \times 10^{-6}$ | 1·43 ± 0·09** |
| Noradrenaline (5) | $10^{-6}$ | 0·98 ± 0·03 |
| Tyramine (4) | $10^{-6}$ | 0·96 ± 0·04 |
| Dopamine (4) | $3 \times 10^{-5}$ | 0·99 ± 0·05 |
| Bradykinin (6) | $3 \times 10^{-7}$ | 0·96 ± 0·08 |

† Response ratio: mean ± s.e.
Significant differences from the control: * $P$ <0·05, ** $P$ <0·01.

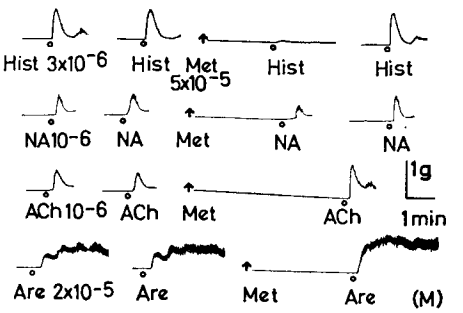


FIG. 1. Effects of methysergide (Met) on the contractile responses to histamine (Hist), noradrenaline (NA), ACh and arecoline (Are). The intervals between administration of test agonists was 20 min. During this period, the preparation was washed with the normal Tyrode solution. Methysergide was introduced into the bath 5 min before the agonist.

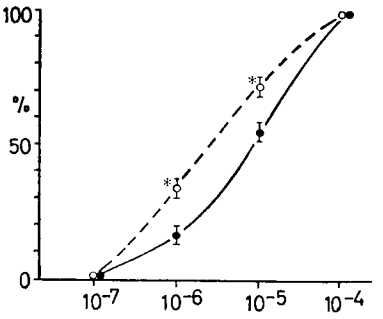


FIG. 2. Concentration-response curves for ACh in the absence (●—●) and presence (○ - - - ○) of methysergide ($5 \times 10^{-5}$ M). Ordinate: percent from the maximum response to ACh. Points: mean values of 4 experiments. Bars: standard errors. * denotes significant difference, $P$ <0·05. Ordinate: % response. Abscissa: concentration of ACh (M).

## DISCUSSION

Methysergide ($5 \times 10^{-6}$ M) potentiated the contractile responses of vas deferens to acetylcholine-like agents but not those to NA-like agents and other drugs used. At a higher concentration of methysergide ($5 \times 10^{-5}$ M), the responses to ACh and arecoline were also enhanced whereas those to NA and histamine were markedly suppressed as reported with other tissues (Dalessio et al 1962; Apperley et al 1976).

The potentiation of the contractile response to ACh ($10^{-6}$ M) by methysergide was not inhibited by

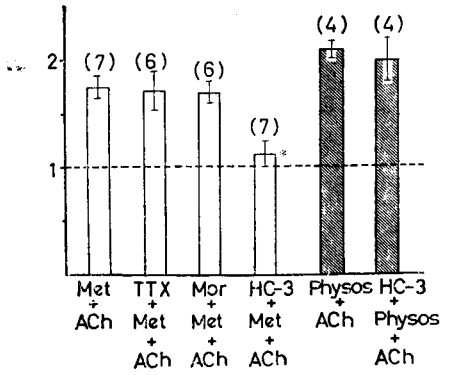


FIG. 3. Influences of TTX, morphine, and HC-3 on the methysergide- and physostigmine-induced potentiation in the contractile response to ACh. Drugs: TTX ($3 \times 10^{-7}$ M), morphine (Mor, $3{\cdot}5 \times 10^{-5}$ M), HC-3 ($10^{-4}$ M), methysergide (Met, $5 \times 10^{-5}$ M) and physostigmine (Physos, $2{\cdot}3 \times 10^{-7}$ M). For methodology, see the text. Ordinate: response ratio to the control (ACh, $10^{-6}$ M). Numbers in parentheses represent the number of experiments. * denotes significant difference, $P \leq 0{\cdot}01$ vs Met plus ACh.

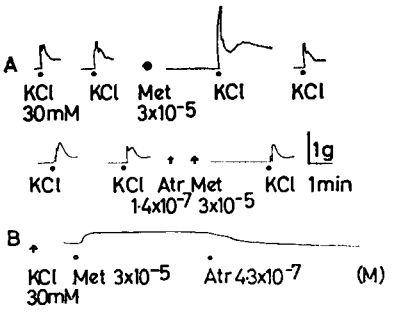


FIG. 4. Effects of methysergide on the $K^+$-induced contraction before and after atropine. In panel A, the interval between KCl administration was 20 min. Atropine (Atr) and methysergide (Met) were added into the bath 10 min and 5 min before KCl, respectively. At the end of each trace, the preparation was washed with the normal Tyrode solution. In panel B, methysergide was added 5 min after KCl.

pretreatment with TTX ($3 \times 10^{-7}$ M) or morphine ($3{\cdot}5 \times 10^{-5}$ M) but was prevented by pretreatment with HC-3 ($10^{-4}$ M). From the negative results obtained with TTX, a blocker of nerve conduction (Gershon 1967), the methysergide-induced potentiation is not mediated through the nerve excitation. Lysergic acids such as methysergide and LSD-25 were postulated to exert a stimulating action on the vascular 5-HT receptor in man (Gant & Dyer 1971) or sheep umbilical vein (Dyer & Gant 1973), dog saphenous vein (Apperley et al 1976), and rabbit ear artery (Apperley et al 1977). In the guinea-pig isolated ileum, 5-HT contracted the longitudinal muscle partly by increasing the output of ACh from the cholinergic nerve terminals, this contraction being suppressed by morphine (Gaddum & Picarelli 1957).

However, it is unlikely from the present results that the potentiation is due to a stimulating action of methysergide on the morphine-sensitive terminal axon.

It is widely accepted that HC-3 is a specific blocker of ACh synthesis in the nerve terminals, which results from inhibiting choline transport across the axonal membrane, and is, therefore, an important tool for distinguishing between direct and indirect action of cholinomimetic agents (MacIntosh et al 1956; Hukovic et al 1965; Katsuragi & Furukawa 1979). Accordingly, the present result with HC-3 implies that methysergide seems to exert an effect at the presynaptic site, presumably by affecting ACh release.

Our interpretation of this potentiating phenomenum is further supported by the facts that the phasic contraction by 30 mM KCl was enhanced, as with ACh, by methysergide ($3 \times 10^{-5}$ M) but this enhancement was prevented by pretreatment with atropine. Furthermore, the continuous tonic contraction elicited by methysergide after treatment with KCl was also inhibited by atropine. Therefore, a depolarization by the high concentration of $K^+$ in conjunction with methysergide may synergistically act on the release of ACh from the cholinergic nerve terminals.

Glegg & Turner (1971) in an in vitro study on human red cells reported that methysergide manifested a slight anticholinesterase activity. However, the physostigmine-induced enhancement of contractile responses of the vas deferens to ACh was not prevented by pretreatment with HC-3. From this fact, the potentiation by methysergide seems not to involve cholinesterase inhibition.

Thus, the present findings provide evidence that the selective potentiation in responses to ACh-agents is due to a facilitation of ACh release from the cholinergic nerve endings by methysergide and that the stripped guinea-pig vas deferens is a useful tissue for investigating an indirect parasympathomimetic action.

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## REFERENCES


Apperley E., Humphrey, P. P. A., Levy, G. P. (1976) Br. J. Pharmacol. 58: 211–221

Apperley, E., Humphrey, P. P. A., Levy, G. P. (1977) Ibid. 61: 465

Birmingham, A. T. (1966) Ibid. 27: 145–156

Dalessio, D. J., Camp, W. A., Goodell, H., Chapman, L. F., Zileli, T., Ramos, A. O., Ehrlich, R., Fortuin, Mck., Wolff, H. G. (1962) World Neurol. 3: 66–72

Dyer, D. C., Gant, D. N. (1973) J. Pharmacol. Exp. Ther. 184: 366–375

Furness, J. B., Iwayama, T. (1972) J. Anat. 113: 179–196

Gaddum, J. H., Picarelli, Z. P. (1957) Br. J. Pharmacol. 12: 323–328

Gant, D. W., Dyer, D. C. (1971) Life Sci. 10: 235–240

Gershon, M. D. (1967) Br. J. Pharmacol. 29: 259–276

Glegg, A. M., Turner, P. (1971) Arch. Int. Pharmacodyn. Ther. 191: 301–309

Hukovic, S., Rand, M. J., Vanov, S. (1965) Br. J. Pharmacol. 24: 178–188

Katsuragi, T., Furukawa, T. (1979) Arch. Int. Pharmacodyn. Ther. 237: 150–159

MacIntosh, F. C., Birks, R. I., Sastry, P. B. (1956) Nature (London) 178: 1181

Metcalfe, H. L., Turner, P. (1970) Arch. Int. Pharmacodyn. Ther. 183: 148–158

Thoa, N. B., Maengwyn-Davies, G. D. (1968) J. Pharm. Pharmacol. 20: 873–876

Watanabe, H. (1969) Acta Anat. Nippon (In Japanese) 44: 189–202