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The effect of ergot alkaloids ergosinine, dihydroergosine and dihydroergotamine on neurotransmission and contractility of the isolated ileum of the guinea-pig

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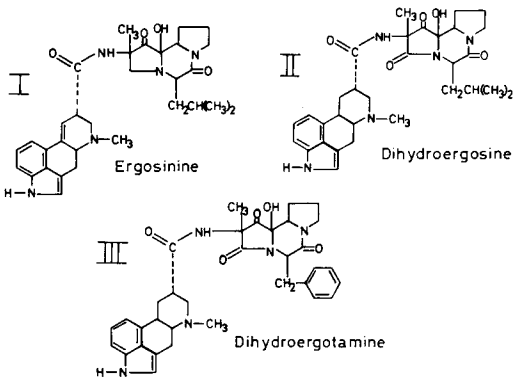
The effects of ergosinine (ESNN), dihydroergosine (DHESN) and dihydroergotamine (DHE) on contractions of the isolated terminal and middle segments of the guinea-pig ileum were studied in-vitro. Responses to cholinergic (3 Hz) and adrenergic stimulation (30 Hz in the presence of atropine) were inhibited, albeit at high concentrations of all three alkaloids (1–30 μg ml^{-1}). Cholinergic neurotransmission was surprisingly more affected than adrenergic transmission. Noradrenaline (NA) contractions, however, were inhibited at very low concentrations (1–30 ng ml^{-1}) with the following order of potency: DHESN = DHE > ESNN. Prazosin was equally as potent as DHESN in inhibiting NA contractions and similarly potent in inhibiting responses to adrenergic stimulation. ESNN, DHESN and DHE when used at concentrations from 1–30 μg ml^{-1} were also found to inhibit 5-hydroxytryptamine > histamine > acetylcholine > KCl contractions. The results suggest that the principal pharmacological action of ESNN, DHESN and DHE on the guinea-pig isolated ileum is the antagonism to NA on the postsynaptic and extrajunctional population of α-adrenoceptors. The neurotransmission, adrenergic as well as cholinergic, appeared to be inhibited via a non-specific presynaptic mechanism presumably regulating the transmitter release. Anti-5-hydroxytryptamine, anti-acetylcholine and antihistamine actions were obtained at similar and relatively high concentrations, thus pointing to a non-specific depressant action upon a common mechanism regulating the contractility of the smooth muscle. Finally, ESNN although a derivative of (+)-isolysergic acid (derivatives of which are generally regarded as inactive) was shown to possess a pharmacological activity comparable to DHESN, i.e. to the drug representing active derivatives of (+)-lysergic acid.

The gastrointestinal pharmacology of ergot alkaloids has not been studied as extensively as the other aspects of their pharmacological profile, and relatively few data are available. Ergotamine has been shown to increase peristalsis and the rate of transport of solid material along the gastrointestinal tract in animals and man, and the doses which were ineffective when used alone could markedly potentiate the stimulating effects of neostigmine (Nickerson 1970). Not long ago, dihydroergotamine (DHE) was used in the complex treatment of the postoperative atonia of the gut (Theisinger et al 1978) but its contribution to the clinical effect has proved difficult to evaluate. In contrast, DHE was also found to inhibit hypermotility of the distal large intestine (Lechin et al 1977).



The alkaloids we have used in the present study were two derivatives of ergosine: (I) ergosinine (ESNN) derived from (+)-isolysergic acid with the (α)5*R*,8*R*-configuration, and (II) dihydroergosine (DHESN) derived from (+)-lysergic acid with the (β)5*R*,8*R*-configuration, and one derivative of ergotamine, i.e. DHE (III) having (β)5*R*,8*R*-configuration. ESNN differs stereochemically from DHESN and DHE in the configuration on position 8 (i.e. in the ring D) of the ergopeptine structure (Rutschmann & Stadler 1978). ESNN was selected for investigation to further evaluate our recent data indicating that the derivatives of (+)-isolysergic acid are also pharmacologically active (Ocvirk & Djordjević, unpublished). Until recently, only derivatives of (+)-lysergic acid were thought to be of pharmaco-

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logical importance (Berde & Stürmer 1978).

Ergot alkaloids and their dihydroderivatives have a great affinity for adrenoceptors, where they behave as agonists, partial agonists (or partial antagonists) and as pure antagonists. The populations of receptors to which they bind differ from organ to organ and the affinity and efficacy (intrinsic activity) vary with their structure and configuration (Rutschmann & Stadler 1978). The initial examination of pharmacological profiles has shown that both ESNN and DHESN act bimodally: they facilitate the spontaneous pendular activity of the isolated rabbit jejunum at low concentrations but inhibit it when used in concentrations above 10 $\mu g\,ml^{-1}$. In addition, both isomers were found to inhibit noradrenaline (NA)-induced relaxation of the rabbit jejunum, sharing the action of DHE but appearing significantly more potent (Radulović et al 1983).

MATERIALS AND METHODS

Terminal and middle segments of ileum were dissected from guinea-pigs, 300–600 g, which had been stunned and bled out. About 2–3 cm long pieces of ileum were set up in a 10 ml organ bath containing Tyrode solution of the following composition (mM): NaCl 137, KCl 2·67, $NaHCO_3$ 11·9, $CaCl_2$ 1·82, $MgCl_2$ 0·11, NaH_2PO_4 0·42 and glucose 5·56. The temperature was maintained at 37 °C and the solution was bubbled with 95% oxygen and 5% carbon dioxide. Contractions were recorded via an isometric transducer and a potentiometric recorder (Basile). Electrical stimulation (ES) was performed via coaxially placed electrodes with trains of pulses delivered from a Grass S8 electronic stimulator. At a frequency of 30 Hz, pulses of 0·3 ms duration were used in trains of 1 s duration. The trains were spaced by 100 s intervals. The stimulus strength was 30–60 V. Experiments with 30 Hz were performed on the terminal ileum segments in the presence of atropine (1 $\mu g\,ml^{-1}$). For ES at 3 Hz, segments of the middle ileum were used. Pulse duration, voltage and the intervals between trains were as described above, only the duration of trains was increased to 2 s.

In experiments with ES the ergot alkaloids were added into the organ bath according to the cumulative method, allowing each concentration 5 min contact with the preparation; within that period three ES-induced contractions were recorded and their magnitude monitored.

In experiments with NA as an agonist the terminal ileum was used, whereas with other directly acting agonists, segments of the middle ileum were used according to the method of Magnus. The experiments were performed in 5–10 min cycles. The ergot alkaloids were allowed a contact period of 3 min duration, while the agonists were kept 20–30 s in contact with the preparation.

The results were tested for significance using Student's *t*-test. The values are the means ±s.e.

Drugs used were: dihydroergotamine mesylate, dihydroergosine mesylate, ergosinine mesylate, cyproheptadine chloride (Lek), acetylcholine chloride (ACh), atropine sulphate, noradrenaline bitartrate (Serva), chlorpyramine chloride (Ciba), histamine dihydrochloride (Koch-Light), 5-hydroxytryptamine (5-HT) maleate (Merck) and potassium chloride (Zorka).

RESULTS

Inhibition of adrenergic neurotransmission and anti-NA action

ES at 30 Hz was applied to segments of terminal ileum, where it is known to produce contractions of adrenergic origin which are resistant to atropine and are selectively blocked by guanethidine and phentolamine (Kažić 1975). All alkaloids used in this study inhibited the neurogenic adrenergic responses within a concentration range of 1–30 $\mu g\,ml^{-1}$. The most potent in this respect was DHESN with the ID50 = 6 $\mu g\,ml^{-1}$. DHESN appeared to be 5 times more potent than DHE and presumably 10 times more potent than ESNN (Fig. 1, right panel).

Terminal ileum of the guinea-pig is contracted in response to exogenous NA which activates α_1-adrenoceptors (Wikberg 1979; Bauer & Kuriyama 1982). As shown in Fig. 1 left panel, DHESN was found to be equipotent with DHE, while ESNN was about 10 times weaker. Thus, the ID50 was about 6 $ng\,ml^{-1}$ for DHESN and DHE and

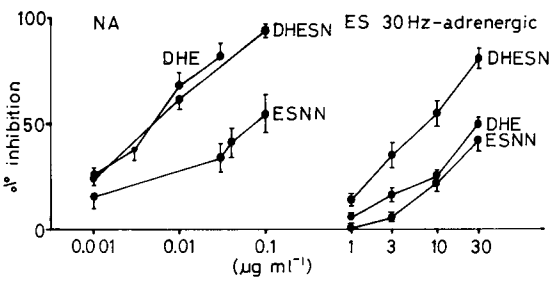

FIG. 1. The effect of ESNN, DHESN and DHE on adrenergic neurotransmission (ES 30 Hz) and NA contractions of the terminal ileum of the guinea-pig. Ordinate: inhibition of control contractions, in percentages. Given are the means ± s.e. of the mean; n = 6–8. Abscissa: concentrations of the ergot alkaloids.

60 ng ml^{-1} for ESNN in experiments with NA-induced contractions, compared with ID50s of 6 μg ml^{-1} for DHESN, 33 μg ml^{-1} for DHE and 60 μg ml^{-1} for ESNN in experiments with ES-induced contractions. The differences between the concentrations of the ergot alkaloids inhibiting ES-induced contractions and those inhibiting NA contractions were striking, within a range of three orders of magnitude. This observation is not novel. However, it again raises questions regarding the site of action and the underlying mechanisms.

In order to localize more accurately the action of ergot alkaloids, two series of experiments with prazosin were performed. This drug is known as a potent α-adrenoceptor antagonist acting solely on the postsynaptic α_1-adrenoceptor subtype. In the first series, prazosin was found to inhibit NA contractions at very low concentrations, with the ID50 = 4×10^{-9} g ml^{-1}. This effect was almost identical in potency to that of DHESN, as shown in Fig. 2. In the second series prazosin was found to act as a rather weak antagonist of the ES-induced contractions. The ID50 was 1×10^{-5} g ml^{-1}, which is more than three orders of magnitude higher than that required for NA contractions, as in Fig. 2.

Inhibition of cholinergic neurotransmission and anti-ACh action

In experiments performed on segments of the middle ileum by means of a cumulative method, all three ergot alkaloids were found to progressively inhibit the neurogenic contractions produced by ES at 3 Hz. As shown in Fig. 3, this inhibition was concentration-dependent within a range of 1–30 μg ml^{-1}. ESNN and DHESN might be regarded as equipotent and both were significantly more potent than DHE when concentrations of 3, 10 and 30 μg ml^{-1} are compared ($P < 0.05$–0.01). The estimated ID50s showed that ESNN and DHESN were three times more potent than DHE in this respect.

In order to localize the observed anticholinergic properties of ESNN, DHESN and DHE, their effects on ACh contractions were studied. The results shown in the right panel of Fig. 3 show that ACh contractions were significantly less sensitive to the inhibitory action of the ergot alkaloids than the neurogenic contractions elicited by ES at 3 Hz. Again, ESNN and DHESN were found equipotent (except at the highest concentrations) and both more potent than DHE. A clearcut quantitative difference was found in the inhibitory potency between the inhibition of ACh contractions and ES-induced contractions. It can be estimated from Fig. 3 that ID50s for ACh contractions for all three ergot alkaloids were about 10 or more times higher. Finally, the observed anticholinergic activity of ergot alkaloids was compared with atropine as a standard anticholinergic drug. ESNN and DHESN were found to be about 3–4000 times less potent.

Inhibition of drug-induced contractions

A series of agonists used comprised substances known to activate smooth muscle via different mechanisms. Some activate receptors which are partly sensitive to ergot alkaloids (5-HT), others activate receptors largely unaffected by the ergot alkaloids studied so far (histamine, ACh), and KCl was used as an agonist producing contractions independent of receptor activation.

The effects of ESNN, DHESN and DHE on NA and ACh contractions are shown in Figs 1–3. Their effects on 5-HT, histamine and KCl contractions are shown in Fig. 4. The pattern of the inhibitory action and the concentrations required were similar. 5-HT

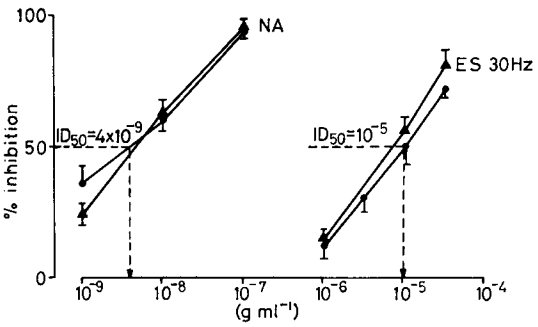

FIG. 2. Comparison with prazosin (●) of the inhibitory action of DHESN (▲) on adrenergic neurotransmission (ES 30 Hz) and NA contractions of the terminal ileum of the guinea-pig. Ordinate: inhibition of control contractions, in percentages. Given are the means ± s.e. of the mean: n = 5–6. The ID50 values are for prazosin only. Abscissa: concentrations of prazosin and DHESN.

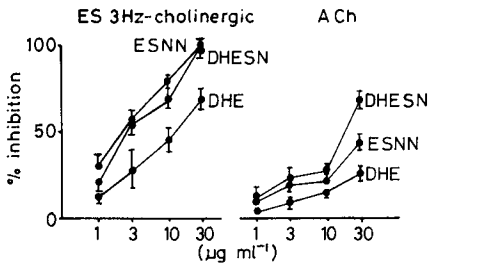

FIG. 3. The effects of ESNN, DHESN and DHE on cholinergic neurotransmission (ES 3 Hz) and ACh contractions of the middle ileum of the guinea-pig. Ordinate: inhibition of control contractions, in percentages. Given are the means ± s.e. of the mean; n = 6–8. Abscissa: concentrations of the ergot alkaloids. Note that the same concentrations were used in both series.

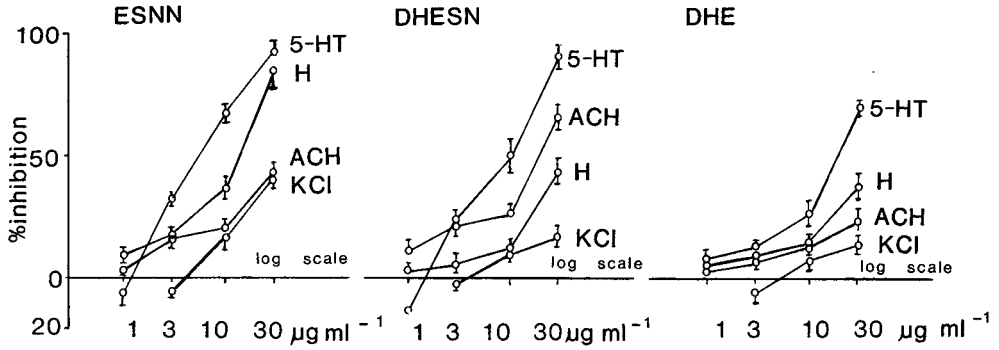

FIG. 4. The effects of ESNN, DHESN and DHE on 5-HT, histamine and KCl contractions of the middle ileum of the guinea-pig. ordinate: inhibition of control contractions, in percentages. Given are the means ± s.e. of the mean; n = 6–8. Abscissa: concentrations of the ergot alkaloids.

contractions were most readily inhibited, while KCl-induced contractions were found to be the most resistant. Histamine contractions were significantly more affected by ESNN than by DHESN and DHE. The statistical analysis of the present data showed that the anti-5-HT action of both ESNN and DHESN was significantly stronger than the antihistamine and anticholinergic action ($P < 0{\cdot}05$–$0{\cdot}01$). Therefore, it was interesting to compare this action of ergot alkaloids with a standard anti-5-HT drug. The results in Fig. 5 indicate that cyproheptadine is about 100 times more potent than ESNN, DHESN and DHE.

In an analogous series of experiments, the observed antihistamine action of ESNN was compared with chlorpyramine. The results in Fig. 6 indicate that ESNN is an antihistamine about five orders of magnitude weaker than chlorpyramine.

DISCUSSION

The principal feature in the pharmacological profile of ESNN, DHESN and DHE obtained in the present

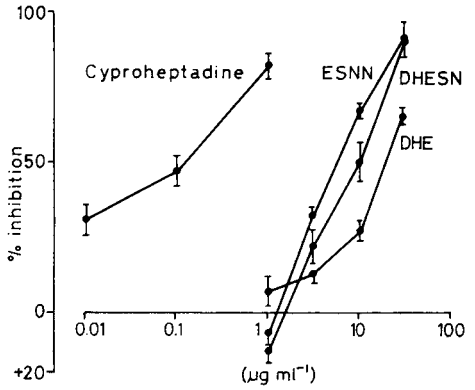

FIG. 5. Comparison with cyproheptadine of the anti-5-HT action of ESNN, DHESN and DHE. Ordinate: inhibition of control contractions, in percentages. Given are the means ± s.e. of the mean; n = 6. Abscissa: concentrations of cyproheptadine and the ergot alkaloids.

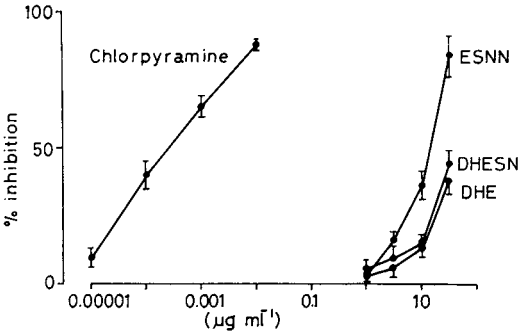

FIG. 6. Comparison with chlorpyramine of the antihistaminic action of ESNN, DHESN and DHE. Ordinate: inhibition of control contractions, in percentages. Given are the means ± s.e. of the mean; n = 6. Abscissa: concentrations of chlorpyramine and the ergot alkaloids.

study was their selective anti-NA action: DHESN appeared to be at least equipotent with DHE, while ESNN was about 10 times less potent than both, but still active in nanomolar concentrations. In a recent paper (Radulović et al 1983) ESNN and DHESN (10 μg ml^{-1}) have been shown to antagonize the relaxation of the rabbit jejunum induced by NA. In the present study, however, to inhibit NA contractions of the terminal ileum of the guinea-pig about 1000 times lower concentrations were sufficient (ID50s of 6–60 ng ml^{-1}). Two comments should be made at this point: (1) ESNN, although a derivative of (+)-isolysergic acid, derivatives of which are generally regarded as inactive and poorly active (Berde & Stürmer 1978), was found to be a potent anti-NA agent, and (2) DHESN was significantly more potent than ESNN as an anti-NA agent, an effect assumed to be due to a blockade of postsynaptic α-adrenoceptors.

An intriguing result of the present study is the relatively weak potency of ergot alkaloids in their inhibition of the neurogenic contractions of adrener-

gic origin produced by ES. The finding that 1000 to 10 000 times higher concentrations of alkaloids were required to inhibit the ES-induced responses than those elicited by exogenous NA raises several questions. In addition, the effects of cholinergic stimulation (ES 3 Hz) were regularly found to be more readily inhibited than the responses to adrenergic stimulation (ES 30 Hz in the presence of atropine). The inhibition of either adrenergic or cholinergic neurotransmission was obtained with high concentrations of alkaloids (1–30 $\mu g\,ml^{-1}$), which were also found to inhibit contractions to a series of agonists. Therefore, the present results indicate that the junctional α-adrenoceptors in the terminal ileum are either completely inaccessible to ergot alkaloids or so different from the extrajunctional receptors that they cannot be recognized and occupied. Alternatively, the inhibition of adrenergic (and also cholinergic) neurotransmission could be non-specific and unrelated to the α-adrenoceptor occupation but due to a general depressant action on the cell membrane of both the nerve endings and the smooth muscle. At micromolar concentrations ergot alkaloids might reduce calcium availability (Kažić et al unpublished results).

The common observation that considerably higher concentrations of α-adrenoceptor blocking agents (including ergot alkaloids) may be required to abolish the responses to nerve stimulation than those to exogenous NA has not been satisfactorily explained so far. There is a possibility that a high local concentration of the transmitter (NA) within the junction between the nerve ending and the effector cell could provide the basis for the relative resistance of the nerve stimulation-induced contractions to the α-adrenoceptor blockade. This explanation, however, could be seriously questioned by the fact that the concentration of NA used to contract the terminal ileum was comparatively high. Namely, the estimated junctional concentration (in the vascular smooth muscle—Suzuki 1981) is of the same order of magnitude as NA bath concentrations used in the present experiments.

The ergopeptine derivatives have been shown to act predominantly on post-synaptic α-adrenoceptors (Loew & Müller-Schweinitzer 1979), and this view was supported by the present results with ESNN, DHESN, DHE and prazosin. These observations obviate the possibility of explaining the observed disparity by a blocking action on the presynaptic α-adrenoceptors and subsequently enhanced release of NA during ES, according to the presynaptic receptor theory (Langer 1974; Starke 1977).

The existence of two pharmacologically distinct populations of α-adrenoceptors was firstly proposed by Hotta (1969) in order to explain the disparity of actions of α-antagonists on the exogenous and nerve-released NA in the vas deferens; 'extrajunctional' receptors would correspond to classical α-adrenoceptors and 'junctional' receptors, activated by the neurally-released NA, were insensitive to α-adrenoceptor antagonists. Recent electrophysiological research (Hirst & Neild 1981) has disclosed two populations of excitatory α-adrenoceptors in the arteriolar smooth muscle: one mediating localized contraction at the NA source, unassociated with neurotransmission and sensitive to phentolamine, and the other mediating the responses to sympathetic stimulation, being associated with a depolarization similar to the excitatory junctional potential and being resistant to α-antagonists. The present experiments on the terminal ileum of the guinea-pig with ESNN, DHESN, DHE and prazosin lend further support to the view favouring two distinct populations of α-adrenoceptors. The extrajunctional or traditional α-adrenoceptors seem to belong to the prazosin sensitive α_1-subtype. The junctional α-adrenoceptors in the terminal ileum, those activated by the neurally released NA during ES, may belong to a distinct population of α_1-adrenoceptors similar to γ-receptors described in some vascular tissues (Itoh et al 1982). They presumably do not belong to the α_2-subtype of adrenoceptors because in the intestinal smooth muscle they mediate inhibitory responses (Bauer & Kuriyama 1982; Wikberg 1979).

It would be precipitate to propose any physiological relevance of the present data to the functioning of the terminal ileum, the sphincter-like portion of the intestine, as the pharmacology of adrenoceptors is still far from being settled (Starke & Docherty 1982; McGrath et al 1982).

Anticholinergic action

Ergot alkaloids neither stimulate nor block ACh receptors. The anticholinergic action of two derivatives of ergosine, ESNN and DHESN, as well as that of DHE, which was observed in the present study, did not appear to be related to a blockade of cholinergic receptors in the intestinal smooth muscle, but, more likely, to be due to a presynaptic action. Cholinergic nerve endings might be more sensitive to a general depressant action of the ergot alkaloids than the smooth muscle cells. The finding that the ID50 for responses to ES at 3 Hz was 10 times smaller than for responses to exogenous ACh

points to a presynaptic site of action. The analysis according to the method of Mayer et al (1981) showed that 70–85% of the anticholinergic action of the ergot alkaloids was presynaptic, i.e. due to a reduced ACh release, and only 15–30% could be accounted for by the effects on the smooth muscle. The experimental comparison with atropine strongly supports this conclusion.

Anti-5-HT action

A common feature for all three alkaloids was the anti-5-HT action, which was always more potent than the respective anticholinergic and antihistaminic actions. The anti-5-HT action of ergopeptine alkaloids is usually less selective than that induced by the amide derivatives of lysergic acid because peptide alkaloids might also combine with α-adrenoceptors when they are used as 5-HT blocking agents (Müller-Schweinitzer & Weidmann 1978). In the intestinal smooth muscle, DHE was found to inhibit 5-HT and histamine contractions in the same concentration ranges (Müller-Schweinitzer & Weidmann 1978) and the results of the present study with ESNN, DHESN and DHE are in accord with a number of previous observations.

Low concentrations of the ergosine derivatives ESNN and DHESN, but not of DHE, appeared to act as coagonists on 5-HT receptors and to potentiate 5-HT contractions. Some enhancement of 5-HT effects has been observed previously in the isolated rat duodenum in the presence of ergotamine (Levi & Michel-Ber 1956). Compared with the inhibition of KCl contractions, 5-HT-induced contractions in the present study were more selectively antagonized by the ergot alkaloids than the responses to any other agonist except NA. However, when compared with cyproheptadine, the anti-5-HT action of the derivatives of ergosine and DHE must be regarded as weak, because their action was about 200 times less potent.

Antihistamine action

Generally, ergot alkaloids do not affect histamine receptors if used in therapeutic concentrations. However, many ergot compounds including DHE have been shown to inhibit histamine contractions in extremely high concentration (from 4–50 μl) (Gaddum & Hameed 1954; Gaddum & Picarelli 1957). The lower segment of this range was used in the present study on the terminal and middle ileum of the guinea-pig with ESNN, DHESN and DHE. Although ESNN was found to be significantly more potent than DHESN and DHE, its antihistamine properties still remain within the non-specific actions because rather high concentrations of the drug were required to inhibit histamine contractions. The finding that chlorpyramine, a conventional antihistamine, was 100 000 times more potent than ESNN and DHESN made their antihistaminic activity negligible and presumably non-specific.

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