Pharmacological differences of non-adrenergic inhibitory response and of ATP-induced relaxation in guinea-pig tracheal strip-chains

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The presence of non-adrenergic inhibitory neurons in the guinea-pig tracheal muscle has been demonstrated by Coburn & Tomita (1973) and Richardson & Bouchard (1975), using isolated tracheal strips under isometric conditions. On the other hand, Coleman & Levy (1974) also recognized these neurons using the isolated tracheal tube preparation under isotonic conditions and proposed adenosine 5'-triphosphate (ATP) as a transmitter in these neurons. Previously (Kamikawa & Shimo, 1976), we reported that the inhibitory response to ATP of guinea-pig tracheal strip-chains is antagonized by indomethacin, aspirin and polyphloretin phosphate (PPP) so that this response may be an indirect one mediated by prostaglandin (PG) E_2. The present report thus describes the influences of these drugs on the non-adrenergic inhibitory response to field stimulation of tracheal strip-chains of guinea-pigs under isometric conditions.

Male guinea-pigs, 300 to 600 g, were used. The tracheal strip-chain was prepared according to the method described in our previous paper and suspended in a 30 ml organ bath with modified Krebs-Ringer solution (composition mM: NaCl, 120; KCl, 4.7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 25; KH_2PO_4, 1·2; glucose, 14; pH 7·4) aerated with 5% CO_2 in oxygen at 37 °. The preparation was mounted under 0·5 g of initial tension and contracted by an application of histamine 10 μM before every stimulation. The tracheal response was recorded isometrically using a force-displacement transducer coupled to a Recticorder (Nihon Kohden). The field stimulation was with rectangular pulses of varied frequencies from 1 to 50 Hz, pulse duration of 0·3 ms and supramaximal voltage, through bipolar platinum electrodes which were 10 mm apart and connected to a Nihon Kohden stimulator, model SEN 1101. The total number of stimulating pulses was kept constant at 60. For the elimination of parasympathetic components in responses to field stimulation, the Krebs-Ringer solution contained 1 μM atropine sulphate. Drugs used were: adenosine 5'-triphosphate disodium salt (Sigma), histamine dihydrochloride, atropine sulphate (Wako Pure Chem.), guanethidine sulphate (Ciba-Geigy), propranolol hydrochloride, tetrodotoxin, indomethacin (Sankyo), and PPP (AB Leo). Indomethacin was dissolved in 50% ethanol, and all other drugs were dissolved in physiological saline.

Field stimulation of the tracheal strip-chains with the stimulus parameters described above elicited only relaxation which depended on the stimulus frequency. The maximal relaxation was obtained at 50 Hz, when the tracheal tension decreased by 500 to 600 mg. These elicited relaxations seemed to be mostly due to the stimulation of intramural nerve fibres, since these were almost abolished by tetrodotoxin 0·1 μg ml^{-1}, as shown in Fig. 1. However, pretreatment with 10 μM guanethidine, an adrenergic neuron blocker, depressed responses only 10 to 15% (Fig. 1). So it is suggested that the elicited relaxation is mainly due to the stimulation of non-adrenergic inhibitory nerve fibres. Propranolol (1 μM), a β-adrenoceptor-blocker, also depressed these responses to a similar degree to guanethidine. Although a high dose of propranolol (10 μM) inhibited these elicited relaxations by 60% (Fig. 1), the local anaesthetic action of this drug may be involved (Morales-Aguilará & Vaughan Williams, 1965). Pretreatment with 10 μM indomethacin, an inhibitor of PG biosynthesis, for 30 min did not inhibit the elicited relaxation in any preparations, but rather enhanced slightly at every stimulus frequency (Fig. 1). Similarly, 100 μg ml^{-1}

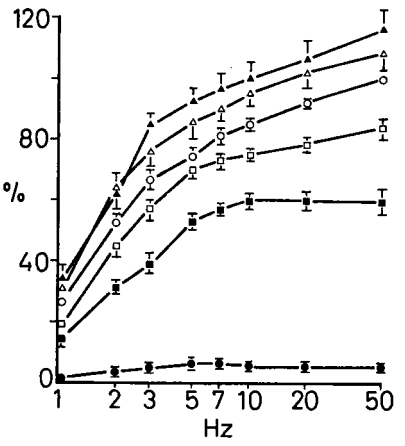

FIG. 1. Effects of ●—● tetrodotoxin (0·1 μg ml^{-1}, n = 20), □—□ guanethidine (10 μM, n = 17), ■—■ propranolol (10 μM, n = 7), △—△ indomethacin (10 μM, n = 12) and ▲—▲ PPP (100 μg ml^{-1}, n = 10) on the frequency-response curve of relaxation obtained by field stimulaton of guinea-pig tracheal strip-chains contracted by an application of histamine (10 μM). ○—○ Control (n = 20). The field stimulation was carried out with rectangular pulses of varied frequencies (1 to 50 Hz), pulse duration of 0·3 ms and supramaximal voltage. The total number of stimulating pulses was kept at 60. Guanethidine, propranolol, tetrodotoxin and PPP were added 5 min before and indomethacin 30 min before an application of histamine.
Abscissa: Stimulus frequencies (Hz) on log scale.
Ordinate: Percentage relaxation (mean ± standard error). 100% = relaxation of control at 50 Hz.

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polyphoretin PPP, a PG antagonist, also enhanced the responses about 15 to 30% and did not show any antagonistic effects. The doses of indomethacin and PPP used here markedly inhibited the relaxation induced by 10 μg ml^{-1} ATP in the presence of histamine, 10 μM, as described in our previous paper.

From these results, it is apparent that the non-adrenergic inhibitory response to field stimulation and the response to ATP of guinea-pig tracheal muscle are different with respect to their pharmacological characteristics. We conclude that the transmitter substance of non-adrenergic inhibitory neurons is neither ATP nor PGs. The enhancement of the inhibitory response by indomethacin and PPP may be caused by inhibition of the PG-mediated negative feedback control of adrenergic neurotransmission (Hedqvist, 1973).

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Actions of amfonelic acid and other non-amphetamine stimulants on the dopamine neuron*

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The mechanism of the central actions of amphetamine has been of great interest, in part because the drug can cause effects in man resembling paranoid schizophrenia and because the drug appears to produce its central actions by releasing and blocking re-uptake of brain catecholamines (e.g. Snyder, Banerjee & others, 1974).

The most striking evidence that amphetamine acts through brain catecholamines is the observation that the drug's central effects are blocked by inhibition of tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis, suggesting that amphetamine acts centrally largely through newly synthesized catecholamine (Weissman, Koe & Tenen, 1966; Sulser, Owens & others, 1968). Consistent with this interpretation, depletion of brain catecholamine pools by reserpine does not inhibit amphetamine's central actions, although peripheral catecholamine depletion is known to inhibit the cardiovascular actions of amphetamine.

Certain other cns stimulants produce amphetamine-like central effects in laboratory animals and in man, but the central effects of some of these are not inhibited by blockade of tyrosine hydroxylase, but are blocked or greatly attenuated by central catecholamine depletion, suggesting a mechanism different from that of amphetamine. Included in this category are cocaine, methylphenidate, and the highly potent stimulant, amfonelic acid (Snyder & others, 1974; Aceto, Harris & others, 1967; Aceto, Botton & others, 1970). All of these drugs produce in the rat a marked cns stimulation, while in man they produce not only an amphetamine-like cns stimulation, but also hallucinations, paranoid ideation and exacerbation of schizophrenic symptoms (Snyder & others, 1974; Rosenberg, F. J., personal communication).

The present study describes experiments on the action of these non-amphetamine stimulants, especially amfonelic acid (AFA). The results indicate that the non-amphetamines have a unique action on the central dopamine neuron that is quite different from that of amphetamine.

The initial indication of a unique action on the dopaminergic neuron came from observations of the effects of AFA on dopamine turnover in the corpus striatum of the rat as measured by the accumulation of the dopamine metabolites, homovanillic acid (HVA) and dihydroxyphenylacetic acid (Dopac). Subsequent studies utilized the disappearance of striatal dopamine after tyrosine hydroxylase inhibition, a useful estimate of impulse flow in the dopamine neuron (Andén, Corrodi & others, 1971). Dopamine, HVA and Dopac were measured in the corpus striatum of drug-treated rats by established fluorometric techniques (Neff & Costa, 1966; Andén, Roos & Werdinius, 1963; Murphy Robinson & Sharman, 1969).

As shown in Table 1, neither AFA nor haloperidol alone affected significantly striatal dopamine concentra-

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