found on average to be 8·3 times more potent than α-MNA. Log dose/response curves are plotted for one experiment (see Fig. 1). This result is in agreement with the findings of other workers. Day and Rand[1] have shown that noradrenaline is 8·5 times more potent than α-MNA on rabbit blood pressure and recently Malik and Muscholl[6] demonstrated that noradrenaline has three times the pressor potency of α-MNA on a rat mesenteric artery preparation.

The finding that α-MNA is equipotent with noradrenaline on cardiac β-receptors yet considerably less potent than noradrenaline on perfused artery preparations supports the idea that the site of the hypotensive action of α-methyldopa is the peripheral vasculature rather than the heart. It is likely that α-MNA is less potent at this site than noradrenaline due to a lesser affinity for α-receptors, the stimulation of which promotes vasal constriction and raises blood pressure.

Substitution on the α-carbon of the phenylethylamine structure such as occurs in α-MNA may diminish its ability to stimulate the α-receptor; this is well known to occur with alkyl substitution on the adjacent amine group.

It must be born in mind that whilst it is valid to compare the effects of α-MNA on α- and β-receptors by perfusion techniques such as are described in the present communication, therapeutically its effect depends upon its liberation at the sympathetic nerve ending.

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Quantitative studies of the release of purine compounds following stimulation of non-adrenergic inhibitory nerves in the stomach

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FOR MANY years it has been assumed that inhibition of intestinal smooth muscle in mammals is mediated solely via the release of noradrenaline from sympathetic neurones. However, since 1964, pharmacological and electrophysiological evidence has been presented of a neurone type in the gut wall which is neither adrenergic nor cholinergic,[1,2] yet causes inhibition of the gut. This neurone is located in Auerbach's plexus; it is the post ganglionic element in a vagal parasympathetic pathway to the

stomach,[3,4] but does not appear to have an extrinsic connection in the colon.[5] Recently, an investigation of the nature of compounds released following stimulation of the non-adrenergic inhibitory nerves was carried out by recycling small quantities of nutrient medium through the vascular system of toad stomachs.[6,7] The results showed that considerable amounts of adenosine and inosine were released during stimulation. On the basis of this and other experiments, it was suggested that ATP or an analogue could be the transmitter substance in non-adrenergic inhibitory nerve fibres to the gut.[6,7] The evidence[7] was found to satisfy broadly the criteria for transmitter substances as laid down by Eccles[8] and included the following observations: (1) ATP or its breakdown products were the only smooth muscle inhibitory compounds released from the gut following stimulation of the inhibitory nerves. (2) Adenine nucleotides caused potent and specific relaxation of smooth muscle preparations containing the non-adrenergic inhibitory nerve fibres. (3) Induction of tachyphylaxis to ATP in rabbit ileum caused a consistent reduction of the responses to stimulation of the non-adrenergic but not of the adrenergic inhibitory innervation. A possible role for ATP as a transmitter in the central nervous system has also been discussed.[9]

Whilst adenosine and inosine were released following stimulation of non-adrenergic inhibitory nerves to the toad stomach, avian Auerbach's plexus, which can be dissected free of underlying muscle and also contains the non-adrenergic nerve fibres, releases AMP following stimulation.[7]

The following questions remain unanswered:

(1) Were the purine nucleosides which were collected from the perfusate, the breakdown products of the neurotransmitter released from non-adrenergic inhibitory nerves?

(2) If these substances are not neurotransmitters or breakdown products of transmitters, what other reasons could there be for purine nucleotide or nucleoside release during nerve stimulation?

In this paper, quantitative studies have been made of the release of nucleosides from the recycled perfused toad stomach preparation and the amounts released during increasing times of perfusion and vagus nerve stimulation are reported. Evidence that the nucleosides released from the toad stomach are metabolites of a single nucleotide is considered.

Isolated stomachs of the toad *Bufo marinus* were perfused with an oxygenated nutrient medium[7] via the cocliac artery for 20 min to remove traces of blood. They were then placed in small chambers and 2·5 ml of nutrient medium was recycled through each preparation for times varying from 5 to 30 min. Stomachs were stimulated via the vagal nerves with 10 pulses/sec at 10 V for 20 sec every minute throughout the period of the recycled perfusion.

Nucleosides were isolated from perfusates by adsorption on activated charcoal and separated from each other by means of paper chromatography as described elsewhere.[7] Adenosine was assayed by the method of Kalcar.[10] Inosine was determined by measuring its specific absorption at 248 mμ relative to the absorption of known standards of inosine which were subjected to the same purification procedure.

Figure 1 shows that adenosine was released from control unstimulated preparations during the first 15 min of perfusion. After this period no further release was observed. It seems unlikely that the

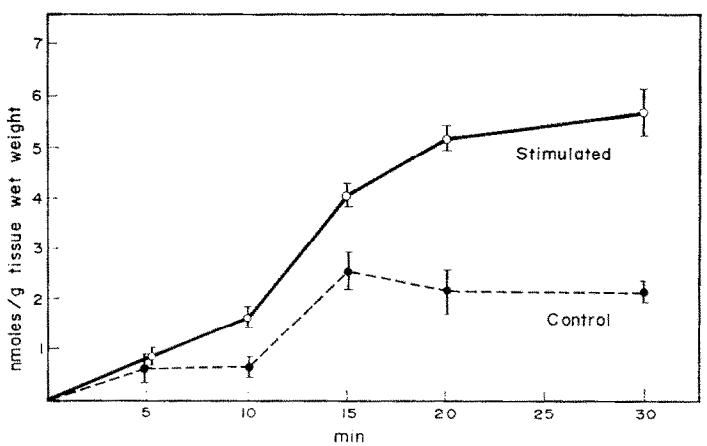

FIG. 1. Release of adenosine from toad stomachs stimulated via the vagosympathetic nerves. Note that stimulation (10 pulses/sec at 10V for 20 sec every minute) caused significant increases in the amounts of adenosine released to the perfusate. Each point represents the mean ± S.E.M. of five determinations.

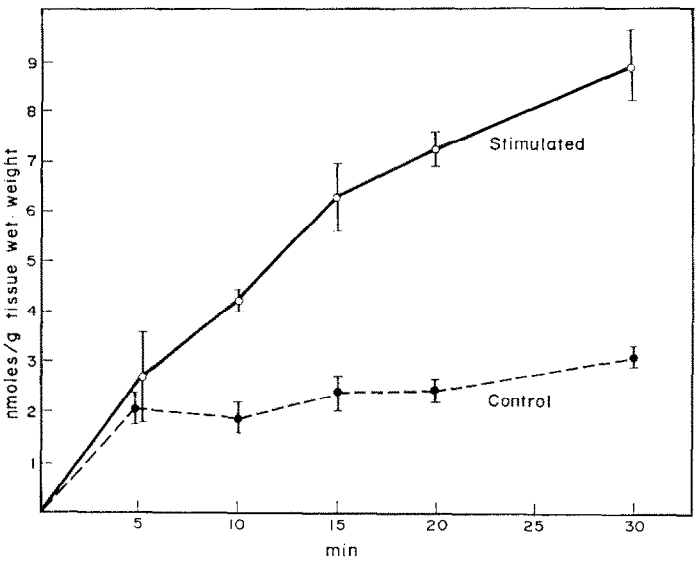

FIG. 2. Release of inosine from toad stomachs stimulated via the vagosympathetic nerves. Note that stimulation (10 pulses/sec at 10V for 20 sec every minute) caused significant increases in the amounts of inosine released to the perfusate. Each point represents the mean ± S.E.M. of five determinations.

adenosine released during the first 15 min comes from traces of blood because each preparation was perfused via the coeliac artery for 20 min prior to the commencement of the recycled perfusion. Stimulation caused a significant increase in the amount of adenosine released to the perfusate. This increase was apparent after 10 min and increased markedly between 10 and 20 min.

In Fig. 2 it can be seen that control unstimulated preparations released inosine during the first 5 min of perfusion only. Stimulation caused a large increase in the amount of inosine released into the perfusate at all times of perfusion. The release of both adenosine and inosine in response to stimulation showed a tendency to decline toward the end of the 30-min period of stimulation and perfusion.

The amount of inosine released in response to stimulation was at least twice the amount of adenosine released during the 5, 10 and 15 min perfusion times and 1·5 times the amount of adenosine released during the 20 and 30 min perfusion times. In view of the findings that adenosine deaminase is present in gut,[11] it is likely that a large proportion of adenosine recycled through the preparation would be converted to inosine.

The finding that non-adrenergic inhibitory nerve stimulation released adenosine and inosine in the above preparation can be reconciled with the suggestion that ATP is the transmitter substance in these fibres if it can be shown that ATP is readily converted to adenosine and inosine in the preparation. To determine whether such a breakdown of ATP does occur in the toad stomach, ATP was added to 2·5 ml of nutrient solution which was recycled through the toad vasculature for 30 min. Figure 3 shows a u.v. photoprint of the chromatographed perfusate. It can be seen that ATP added to the perfusate was converted to adenosine, inosine and a trace of adenine.

Thus the results in this paper suggest that the purine nucleosides collected from the perfusate were the breakdown products of ATP and therefore support and extend the view that ATP may be the transmitter substance released from non-adrenergic inhibitory nerves in the gut. However, the positive identification of a nucleotide as the transmitter substance in non-adrenergic inhibitory nerves requires further evidence, including the discovery of compounds which will specifically modify the actions of the released transmitter substance and produce parallel effects on responses to nerve stimulation and applied nucleotide.

The possibility that the purine nucleotides or nucleosides released from nerves are not neurotransmitter substances but appear for other reasons, must be considered. A variety of functions for ATP in neurones has been suggested. Firstly, it has been claimed that ATP plays a role in the normal physiology of the axonal membrane. Okamoto, Askari and Kuperman[12] and Abood, Koketsu and Miyamoto[13] have suggested that nucleotides combine with protein in the excitable membrane and

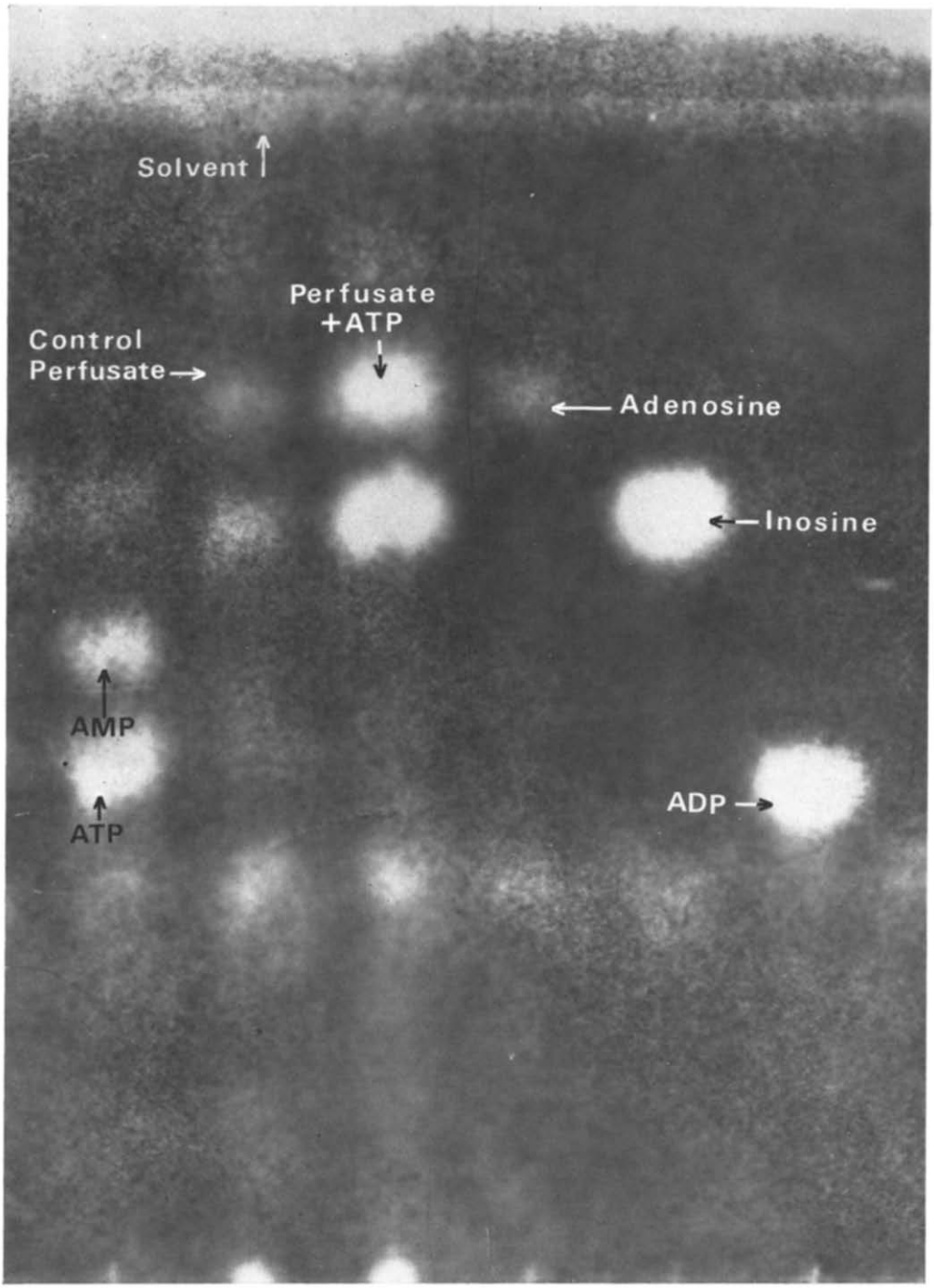

FIG. 3. Breakdown of ATP perfused through toad stomach vascular bed. The figure shows an ultraviolet photoprint of a chromatogram spotted with (from the left) ATP + AMP, control perfusate, perfusate with ATP added before recycling, adenosine, inosine, ADP. Chromatogram run for 12 hr ascending in solvent iso-butyric acid–water–0·880 ammonia–0·1 M EDTA (100:55·8:4:1·6).

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that nerve stimulation causes the release of nucleotides from the membrane. Furthermore, Caldwell and Keynes[14] have proposed that ATP provides phosphate bond energy for sodium extrusion in giant axons. Nachmansohn[15] also mentions a requirement for phosphate bond energy in or near the axonal membrane in order that acetylcholine may be resynthesized for its role in permeability changes of the axonal membrane during activity. Secondly, Holton[16] reported that ATP was liberated from sensory nerve endings following antidromic stimulation. However, this seems to be an unlikely explanation of the effects observed in the gut since a preganglionic efferent innervation of the non-adrenergic inhibitory neurones has been described.[3, 17, 18] Thirdly, ATP is known to have a binding function in the catecholamine containing vesicles of adrenergic neurones in the adrenal medulla.[19] However, Stjärne[20] has been unable to find evidence of ATP release from spleen following stimulation of adrenergic nerves (even though it is now well established that it is released with catecholamines from cells in the adrenal medulla[19]), but the possibility that ATP may have a function in the storage and/or release of other transmitter substances cannot be discounted, despite a lack of supporting evidence.

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Sodium- and potassium-activated ATPase of beef brain—Effects of some tranquilizers

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THE ROLE of sodium- and potassium-activated ATPase (Na^+K ATPase, EC 3.6.1.4) upon active cation translocation across the cellular membrane is well established.[1] A number of central nervous system depressants have been shown to inhibit this enzyme activity.[2–10] Tranquilizing actions of