

DISCUSSION

Rat Ileum

There is considerable evidence, both pharmacological (Lee and Shideman, 1959; West et al. 1966) and biochemical (Bhagat et al. 1967) which suggests that the sympathomimetic effects of nicotine and DMPP are mediated through a release of catecholamines. In the present study, nicotine, DMPP and ACh caused a relaxation of the rat isolated ileum in the presence of hyoscine. The relaxant effect was absent in reserpinised preparations and was restored by NA. This coupled with complete block produced by alpha- and beta-blockers would indicate the mediation of NA release in the relaxant effects of nicotine, DMPP and ACh.

Available experimental evidence does not clearly indicate whether nicotine and DMPP, both powerful stimulants of autonomic ganglia, liberate catecholamines directly from peripheral stores or indirectly by ganglionic stimulation. Since hexamethonium antagonized the

action of nicotine, Kottegoda (1953 b) postulated the existence of sympathetic ganglia in the heart. However, histological examination of the cat papillary muscle on which nicotine exerted a stimulatory effect failed to reveal the existence of ganglion cells (Lee and Shideman, 1959). Though it is possible that ganglionic synapses exist in the periarterial sympathetic nerves of the intestine, an anatomical arrangement known to occur in the sympathetic nerves to the vas deferens of the guinea pig (Bentley and Sabine, 1963; Birmingham and Wilson, 1963), there is no evidence for this. On the other hand, there is good evidence that the sympathetic nerves to the intestine are purely postganglionic (Burnstock, 1969).

If there are no sympathetic ganglia in the intestine, then nicotine, DMPP and ACh might be exerting their effects through stimulation of extra-ganglionic sites (sympathetic nerve endings and chromaffin tissue) (Lee and Shideman, 1959; Wright and Shepherd, 1965; Khan et al. 1965; Muscholl and Vogt, 1964). This peripheral nicotine action is blocked by hexamethonium (Paton and Zaimis, 1952). The

major evidence for this mechanism has been the detection of ACh-induced antidromic discharge in postganglionic C fibres of splenic nerve upon close rapid intra-arterial injection (Ferry, 1963). The assumption is made that this discharge is accompanied by orthodromic impulses which evoke NA release in the same fashion as physiological nerve impulse traffic (Ferry, 1966). This proposal does not seem to be applicable to the results of present experiments, since the ganglion blockers differentially blocked the effects of nicotine and DMPP. The same argument would also exclude the action of ganglion blockers in preventing the entry of nicotine, DMPP and ACh into the nerve endings, a suggestion entertained for ACh (Burn and Gibbons, 1964). Similar results were obtained with cocaine, DMI and procaine which have powerful local anaesthetic action (Iverson, 1968). Further, as pointed out by Ferry (1966), with a rapid intra-arterial injection of ACh, its concentration at the nerve terminals might be expected to rise rapidly leading to a rapid depolarisation and generation of impulse. In vitro experiments with the addition of ACh

to the bathing fluid, rapid high concentration of ACh about the nerve terminals would not be achieved and thus the nerve might accommodate to the slowly rising depolarisation and impulse might not be generated.

Tetrodotoxin blocks propagated action potentials in nerve and muscle by preventing the rapid inward sodium current without itself causing depolarisation of the membrane (Narahashi et al. 1964). In the guinea pig vas deferens hypogastric nerve preparation, tetrodotoxin which blocked preganglionic stimulation did not prevent contraction in response to added NA or ACh (Ozawa and Sugawara, 1967). Krauss et al (1970) observed that in cat isolated spleen, tetrodotoxin prevented the release of NA by sympathetic nerve stimulation and blocked the antidromic discharge produced by intra-arterial administration of ACh but did not abolish catecholamine release induced by ACh. In the present study tetrodotoxin blocked responses to nicotine and DMPP but did not affect those to ACh and potentiated those to NA.

These results point to different loci of action of nicotine and DMPP on the one hand and those of ACh on the other.

Another possibility is that nicotine and DMPP might be exerting their effects through the stimulation of parasympathetic ganglion cells of Auerbach's plexus. Ganglionic synapses are more sensitive to hypothermia than are the postganglionic neurones and the peripheral receptors (Ambache, 1946; Gillespie and Wishart, 1957; Khan et al. 1965). In the present experiments, hypothermia blocked the sympathomimetic effects of nicotine and DMPP but not those of ACh. This coupled with identical results obtained with ganglion blocking agents suggest that nicotine and DMPP may be acting on parasympathetic ganglia while the site of action of ACh is different. Further support for this suggestion is derived from observations with cocaine, procaine and DMI which are all powerful local anaesthetics (Iversen, 1968) and local anaesthetics possess potent ganglion blocking action (Hazard, 1949) and they did indeed act like ganglion blockers in the present experiments.

HC-3 prevents the synthesis of ACh by interfering with the transport of choline to its intraneuronal site of acetylation; this leads to depletion of neuronal ACh and failure of intermittently stimulated cholinergic fibres (MacIntosh et al. 1956; Schueler, 1960). HC-3 blocks responses of cat atria, rabbit ear vessels, cat spleen, guinea pig colon and rabbit and dog nictitating membrane to stimulation of postganglionic sympathetic fibres (Chang and Rand, 1960; Brandon and Rand, 1961; Rand and Ridelagh, 1965; Jacobowitz et al. 1965; Arya and Gulati, 1968). These and other observations led Burn and Rand (1965) to postulate the existence of a cholinergic link between postganglionic sympathetic impulses and the release of NA as the final adrenergic transmitter. In the present study, HC-3 and triethylcholine whose action is similar to HC-3 (Bowman and Hems-worth, 1965) considerably reduced the sympathomimetic effects of nicotine and DMPP; the effects of ACh were, however, potentiated. In this respect these results are in accord with those of Leadres and Long (1962) who

postulated that nicotine stimulates atria by releasing ACh from parasympathetic ganglia and this in turn leads to mobilisation of catecholamines. There is no explanation for the inability of choline to reverse HC-3- or triethylcholine-induced block, in the present study.

Although I did not measure spontaneous release of ACh from the rat ileum, considerable amounts of ACh were released following the administration of nicotine and DMPP. Prior incubation with HC-3 and triethylcholine in concentrations that considerably blocked the sympathomimetic effects of nicotine and DMPP reduced the release of ACh; the reduction was statistically significant with HC-3 but not significant with triethylcholine probably due to large variations in individual observations. Thus evidence in favour of nicotine and DMPP releasing ACh through activation of parasympathetic ganglia is strong.

Increased Ca^{++} concentration is reported to completely reverse inhibition by bretylium of the sympathetic effects of either nerve stimulation on ACh in the rabbit ileum or atria (Burn and Gibbons, 1965; Burn and Welsh, 1967).

In accord with the results of Burn and Gibbons (1965) who demonstrated Ca^{++} dependency of NA release by ACh, it was observed that the sympathomimetic effects of nicotine, DMPP and ACh were absent in Ca^{++} -free medium and that restoration occurred on reintroduction of Ca^{++} into the medium. Further, the neurone blocking action of xylocholine, bretylium and guanethidine is also reversed by dexamphetamine (Day and Rand, 1962; Day, 1962; Gokhale et al, 1965, 1966). In the present experiments, the three neurone blockers blocked responses to nicotine, DMPP and ACh in normal preparations and in reserpinized preparations wherein the sympathomimetic effects had been restituted by prior exposure to NA. In such reserpinized preparations, dexamphetamine partially reversed the neurone blocking action of xylocholine and bretylium but not that of guanethidine. Possibly, the neurone blockers prevent the action of endogenously released or exogenously applied ACh by reducing the availability of Ca^{++} and dexamphetamine reverses the block by enhancing the availability of Ca^{++} . Since guanethidine significantly

reduced the output of ACh by nicotine, it is likely that guanethidine in addition has a ganglion blocking action evidence for which on the rabbit ileum has already been provided from this laboratory (Gokhale et al. 1963).

The results discussed above demonstrate that nicotine and DMPP stimulate parasympathetic ganglia in the rat ileum to release ACh and that this endogenously released ACh or exogenously applied ACh, in turn releases NA and the mechanism of NA release is generally consistent with the proposed role of ACh in sympathetic nerve function (Burn and Rand, 1959).

Rabbit ileum

Stimulation of the splanchnic nerve innervating the intestine of teleost fishes elicits motor response which is blocked by atropine and therefore, the splanchnic nerves are thought to be cholinergic (Young, 1935; Burnstock, 1958). Burn (1968) obtained similar findings on stimulation of periarterial nerves of

intestine of 5 month old chickens. Burn (1968) also reported that stimulation of the periarterial nerves to the intestine of 1 - 8 day old rabbits sometimes elicited motor responses and sometimes inhibitory responses. In intestine from rabbits older than 8 days, only inhibitory responses were elicited. This led him to suggest that a change from cholinergic innervation of the intestine of the trout and chicken to adrenergic innervation of intestine of rabbit takes place during the course of evolution.

In the present study, stimulation of periarterial nerve to intestine of 1 to 3 day old rabbits elicited motor responses at all frequencies (1 - 20 Hz). The responses were potentiated by physostigmine and blocked by hyoscine indicating the release of ACh. These findings are in accord with those reported by Burn (1968).

Boatman et al. (1965) perfused the hindleg vessels of newborn dog (1 - 14 day old). Stimulation of the lumbar sympathetic chain caused a fall in perfusion

pressure despite the fact that blood pressure was very very low, being at 1 day 30 mm Hg; and injection of adrenaline caused a rise in perfusion pressure. In the present experiments, NA (200 - 500 ng/ml) failed to relax the intestine of 1 day old rabbits but preparations from rabbits 2 - 12 day old were relaxed. It seems, that adrenoceptors of the ileum develop on the 2nd day of life.

Following exposure of 1 - 3 day old rabbit ileum preparations to NA (1 µg/ml), nerve stimulated motor responses were abolished at all frequencies, but were not converted to relaxant responses. One explanation for the block of motor responses after exposure to NA may be that NA acts on neuronal alpha-adrenoceptors of periarterial nerves (Kosterlitz et al. 1970; Knoll and Vizi, 1971).

With advancing age, the lower frequencies could elicit relaxations and exposure to NA converted motor responses to inhibitory responses. Possibly, periarterial nerves of younger rabbits contain little or

no NA, but get endowed with this amine as the age advances resulting in the conversion of motor responses to inhibitory responses and potentiation of inhibitory responses at higher frequencies observed after exposure to NA.

An apparently anomalous result was that the endogenous NA content of intestine of 1 - 6 day old rabbits was considerably higher than that of adult rabbit (approximately 200%). The endogenous NA content declined sharply on the 7th day and then declined gradually to the adult level by the 12th day. Ignarro and Shideman (1968 a) observed a high NA content in the egg yolk; this NA migrates to embryonic chick heart on the 4th day, while sympathetic innervation to embryonic chick heart develops on the 5th day of incubation (Romnoff, 1960; Hamilton, 1952; Szepeswol and Bron, 1935 a, b). ^3H - NA injected into the wing vein of fertile hens, is taken up unchanged by the yolk of the developing egg indicating that maternal catecholamines may serve as a source of those found

in the egg yolk (Ignarro and Shideman, 1968 a). Furthermore, enzymatic machinery for the synthesis of NA develops later than the appearance of NA in the egg yolk (Ignarro and Shideman, 1968 b). The high NA content of intestine of 1 - 6 day old rabbits observed in the present study may have been likewise derived from maternal source and stores in extraneuronal sites. The failure of periarterial nerve stimulation to evoke relaxant responses further supports the contention that the storage of NA might be extraneuronal.

A sharp decline in the endogenous NA content occurring on the 5th day of incubation in both the embryo and the embryonic heart of chick has been attributed to sudden enhanced metabolism of catecholamines due to sharp increase in the COMT and MAO activities in liver and heart (Ignarro and Shideman, 1965, 1968 c,d). In the light of this, the sharp decline in the NA content of intestine of 7 day old rabbits observed in the present experiments could be

attributed to sudden enhanced metabolism of NA.

The ability to accumulate NA is dependent on two processes: (i) neuronal uptake and (ii) storage or binding mechanism. The accumulation of exogenously added NA was found to increase gradually from 1 - 6 days. On the 7th day, there was a sudden spurt in the ability of intestine to accumulate NA. The poor ability to accumulate NA in 1 - 6 day old rabbit intestine may either be due to poor development: (i) of neuronal uptake mechanism, or (ii) of binding mechanism, or (iii) of both.

T/M ratios of less than unity indicate passive diffusion (Ignarro and Shideman, 1968 d). Although the T/M ratios of less than unity obtained for 1 - 6 day old rabbits would suggest passive diffusion of NA, the blockade of accumulation of NA by cocaine indicates the possibility that the uptake mechanism may be developed but that the NA binding ability may be poor. That this may have been so, is reflected in the failure of NA to convert motor responses to inhibitory

ones. The small amount of retained NA may have been responsible for the blockade of motor responses in preparations from 1 - 6 day old rabbits.

The ability of intestine from rabbits older than 7 days to accumulate NA was indicated by T/M ratios of 2.96 and above and would suggest that uptake was fully developed from the 7th day onwards. Moreover, despite the apparent fall in NA content, the relaxant responses to periarterial nerve stimulation were obtainable at all frequencies implying that catecholamine synthesizing machinery also becomes fully developed during this period. This conclusion is in accord with the literature report (Burn, 1968 a), that innervation is fully functional on 7th day in rabbit intestine.

Day and Rand (1961) observed that the inhibitory response of 12 day old rabbit intestine to periarterial nerve stimulation at 50 Hz was converted to motor response by guanethidine treatment and that this motor response

was blocked by atropine and suggested the basic cholinergic nature of the nerves. Thus it may be possible, that ACh is released at all frequencies in older preparations too, but, since large stores of NA are built up, ACh releases NA, resulting in inhibitory responses.