CHAPTER - 5

DISCUSSION

5. DISCUSSION

5.1. Splenic and aortic strips

The EC_{50} values computed for NA and Adr were $3.39 \pm 0.25 \times 10^{-9}$ M and $2.29 \pm 0.26 \times 10^{-9}$ M respectively with spleen indicating that Adr is more potent than NA on this tissue. A similar order of potency for the two amines was observed with the cat spleen by Bickerton (1963). The EC_{50} value obtained with goat aorta was $2.14 \pm 0.16 \times 10^{-8}$ M and approximates the value reported with the rabbit aorta (7.5×10^{-8} M) by Kurahashi and Shibata (1971). Phentolamine shifted the concentration-response curves of NA and Adr to the right in a parallel manner and there was no block of maximal responses. These observations suggested competitive antagonism. Detailed work involving pA values was performed with spleen only. The slopes of pA plots with both NA and Adr were not significantly different from the theoretical value of unity for competitive antagonism (Arunlakshana and Schild, 1959) and the pA_2 values of phentolamine with NA and Adr were 8.0 and 8.06 respectively. These values are almost similar to the pA_2 values for this alpha-adrenoceptor blocker reported by Gulati et al., (1968) with rabbit posterior vena cava ($pA_2 = 8.0$) and by Sanders et al. (1975) with rabbit aorta ($pA_2 = 8.01$). Finally, phenoxybenzamine an irreversible competitive alpha-adrenoceptor antagonist (Furchgott, 1954) blocked maximum responses to both the amines in a non-competitive manner. Thus the adrenoceptor of the goat spleen also shares in common the hitherto investigated properties with alpha-adrenoceptor of spleen tissues from other species (Ignarro and Titus, 1968; Ayers et al., 1972). It would, therefore, be fair to conclude that the alpha-adrenoceptor of goat spleen and probably of aorta is similar to that of other tissues.

The potentiating effect of cocaine on responses to Adr has been known for a long time (Frohlich and Loewi, 1910). This potentiating effect is accounted for as due to block of uptake$_1$ of NA and Adr, a unique capacity of sympathetically innervated tissues to concentrate catecholamines from external medium (Iversen, 1968). In the light of this, the failure of cocaine to potentiate responses of goat spleen and aorta to NA demonstrated in the present study appeared surprising. The question posed was " do goat spleen and aorta lack uptake$_1$ process?." In experiments designed to answer this question, results obtained did indeed suggest a lack of uptake$_1$ process in these tissues. For example, the goat spleen and aortic strips incubated with various concentrations of NA for different times did not take up any NA actively and cocaine had no effect in these experiments either. Results of uptake experiments with goat heart were similar to those with spleen and aorta (Table 1). If the NA content of a tissue can be taken as a rough approximation for the sympathetic innervation of that tissue, then 13.72 ± 0.18 nmole/g, 6.8 ± 0.86 nmole/g and 10.24 ± 0.21 nmole/g concentrations of NA for goat spleen, aorta and heart respectively would imply better sympathetic innervation for these tissues than for rat heart whose NA content is 5.74 ± 0.03 nmole/g (Iversen, 1963) and yet there was no evidence for uptake$_1$ process in these tissues. On the basis of similar results obtained with these three goat tissues it would be tempting to extrapolate and suggest that the lack of uptake$_1$ is ubiquitous for all sympathetically innervated goat tissues. The function of uptake$_1$ process is to terminate the action of NA released from sympathetic nerve terminals (Iversen, 1963). In the light of this, it would appear enigmatic as to why the goat spleen, aorta and heart should lack uptake$_1$ particularly since the NA contents of these goat tissues are more than those of rat heart which has considerable uptake$_1$ capacity (Iversen, 1963).

Perhaps mechanisms other than $uptake_1$ control the concentration of NA at the alpha-receptor in the goat tissues. Support for this conclusion is provided by the report of Sheys and Green (1972) who demonstrated that $uptake_1$ is relatively unimportant for modulating the concentration of amines at the receptors in rabbit spleen. The high NA content and lack of potentiating effect of cocaine could also be accounted for by the presence of chromaffin tissue rather than sympathetic nerve endings. This possibility needs further investigation using fluorescent microscopy.

Mouse spleen contains both alpha and beta-adrenoceptors (Ignarro and Titus, 1968) while only alpha-receptors are reported for the spleen of rabbit (Patil et al., 1971; Sheys and Green, 1972), dog (Davies et al., 1969; Takano, 1969) and cat (Greenway and Stark, 1970). Experiments were,therefore, performed to examine if the goat spleen contains only alpha-receptors or both alpha and beta-adrenoceptors. ISO (9×10^{-7} M - 3.6×10^{-6} M) did not produce relaxation of submaximally contracted spleen strip. Thus, the goat spleen does not seem to contain beta-receptors, a conclusion in accord with that of Patil et al., (1971) and Sheys and Green (1972) with rabbit spleen, Greenway and Stark (1970) with cat spleen and Davies et al. (1969)and Takano (1969) with dog splenic capsule. Higher concentrations of ISO (1.63×10^{-5} M - 2.58×10^{-4} M) contracted goat spleen in a concentration-related manner. This result is in agreement with the reported contractile effect of high concentrations of ISO in spleens of cat (Bickerton, 1963; Davidson and Innes, 1970; Kizaki and Abiko, 1966; Leszkovszky and Tardos, 1968), kid (Kizaki and Abiko, 1966; Takano, 1969), rabbit (Jacobi and Fontaine, 1966; Kizaki and Abiko, 1966) and dog (Takano, 1969). Phenoxybenzamine blocked ISO-induced contractions which indicated alpha-receptor action of ISO supporting the conclusions of Ignarro and Titus (1968) with mouse spleen, of Takano (1969) with kid and dog spleens and of Guntheroth and Mullins (1963) with dog spleen.

Several proposals have been advanced from time to time to explain the sympathomimetic actions of tyramine. The most commonly advanced hypothesis is that tyramine acts indirectly by releasing NA from intragranular stores in the sympathetic nerve endings (Goodman and Gilman, 1974). This indirect action of tyramine is also held responsible for tachyphylaxis (Weiner et al., 1962 ; Innes, 1962). Another view is that tyramine acts directly on adrenoceptors (Vane, 1960; Takenaka, 1963; Hudgins and Fleming, 1966; Bevan and Verity, 1967; Gulati and Kelkar, 1971). Since, in the present experiments cumulative response with tyramine could be repeatedly elicited at intervals of 60 minutes, the indirect action can be excluded. The prototype alpha-adrenoceptor blocker, phentolamine blocked responses both to NA and to tyramine suggesting a common site of action for the two amines. These results negate the view that tyramine acts on distinct "tyramine receptors" (Maling et al., 1971; Krishnamurthy and Grollman, 1972; Barnett et al., 1968). Ruffolo et al. (1977) have proposed that imidazoline agonists interact at a different site on the alpha-adrenoceptor than their phenylethylamine counterparts. Since both tyramine and naphazoline lack beta-hydroxyl group on the side chain, they have greater chemical resemblance with each other than with NA. Thus, the site of action of tyramine in goat spleen in relation to the proposal of Ruffolo et al.. (1977) was investigated. For this purpose, desensitization experiments were performed (Gaddum, 1953; Waud, 1968; Schild, 1973). Prior exposure of the tissue to high concentrations of tyramine or naphazoline desensitized it to tyramine and naphazoline but not to NA. On the other hand, prior exposure of the tissue to a high concentration of NA desensitized it to NA but not to tyramine or naphazoline. The possibility is, therefore, strong that tyramine and naphazoline share a common site on alpha-adrenoceptor which is distinct from the NA site. That tyramine may act on sites which are different from the NA site is supported by differential influence of cocaine and reserpine on responses to the two amines. NA was not affected by cocaine and reserpine while the tyramine was blocked.

ACh produced concentration-related contractions which were competitively blocked by atropine suggesting the presence of muscarinic receptors. The pA_2 value of atropine obtained in the present study (pA_2 = 7.6) is somewhat less than that reported (pA_2 = 8.27) in the literature (Schild, 1947). The muscarine receptors appear to be on the capsular smooth muscle (Saad, 1935) since the concentrations of ACh used were high. Physostigmine not only did not potentiate ACh, but blocked it. The lack of potentiation was thought to be due to the absence of acetylcholinesterase in this tissue and confirmation of this idea was obtained in biochemical experiments designed to assay the enzyme in this tissue. Similarly Fillenz (1970) obtained no evidence for AChE in cat spleen by electron-microscopy. Absence of AChE would suggest absence of cholinergic innervation. Support for this conclusion is derived from the observation of Schafer and Moore (1896) who demonstrated that dog and cat spleens were not influenced by vagal fibres.

Innes (1962) described two mechanisms of 5-HT-induced contractions of isolated strips of cat spleen: one directly on receptors for Adr, the second due to release of stored NA which in turn acts on receptors for Adr. In the present study cumulative-concentration response curves for the contractile effects of 5-HT elicited after rest periods of 60 minutes between each pannel of responses showed tachyphylaxis. However, EC_{50} concentrations of 5-HT elicited reproducible contractile responses. Xylocaine which has been shown (Hey and Willey, 1954) to suppress certain responses to sympathetic post-ganglionic nerve stimulation without antagonizing responses to Adr or NA and acts by suppressing the release of transmitter (Exley, 1957), was found in the present experiments to decrease responses to EC_{50} of 5-HT without affecting those to NA. Furthermore, phentolamine blocked responses to both 5-HT and NA but cyproheptadine and reserpine could block responses to 5-HT and not to NA.

The occurence of tachyphylaxis with maximal responses precluded the use of higher doses of 5-HT for studying the effects of blockers. Thus, it is possible that the maximal responses to 5-HT may have been blocked partially with the concentration of phentolamine which blocked EC_{50} responses to 5-HT almost completely. To sum up, the significant findings with 5-HT are the occurence of tachyphylaxis, block of response by xylocaine and phentolamine and differential block by cyproheptadine. These findings suggest that responses to 5-HT may atleast partly be direct on 5-HT receptors and partly through release of NA and support the proposal of Innes (1962) made for the cat spleen. It is possible that 5-HT releases NA by depolarization of sympathetic nerve endings/ chromaffin cells in goat spleen, a proposal already entertained for chromaffin cells of gerbils (Douglas et al., 1967).

Contraction of smooth muscle is dependent upon Ca^{++} (Somlyo and Somlyo, 1968) and different contractile agents differ in eliciting contractile responses by the use of different sources of calcium (Hudgins and Weiss, 1968; Northover, 1968; Van Breeman et al., 1973). For example, contraction with K^{+} is dependent upon extracellular Ca^{++} permeability through membrane depolarization; 5-HT-induced contractile response is partially dependent upon both extracellular and intracellular Ca^{++} while NA-induced contractile response is mainly dependent upon the intracellular Ca^{++} through the release from bound sites (Kalsner et al., 1970; Hudgins and Weiss, 1968; Van Breeman et al., 1972; Krishnamurthy and Grollman, 1976, Hinke, 1965; Jhamandas and Nash, 1967). In the Ca^{++}-free medium containing EDTA (1×10^{-5} M) there was significant inhibition of responses to 5-HT (7.29×10^{-5}M) and KCl (2.69×10^{-2} M). However, responses to NA (7.74×10^{-8} M) and tyramine (5.46×10^{-5} M) were not altered significantly. Thus the present results generally fit in with the requirement of different Ca^{++} pools for K^{+}, NA and 5-HT-induced contractions.

Histamine has been shown to cause contraction of both splenic strips and complete spleens of the cat (Day and Rand, 1963; Innes, 1962; Saad, 1935), dog (Ferguson et al., 1936; Saad, 1935), rabbit (Magee, 1946; Saad, 1935), guinea-pig, rat, pig and ox (Saad, 1935). However, the view that histamine has direct action has not been unanimous. It has been shown that histamine has a dual action in that it releases catecholamines from stores in sympathetic nerve endings and has a direct action on the smooth muscle (Everett and Mann, 1967; Maengwyn, 1968). The possibility of indirect action of histamine through release of adrenergic or cholinergic transmitter could be excluded in the goat spleen because the histamine-induced contractions were not blocked by atropine, hexamethonium and phentolamine. Furthermore, the contractile action of histamine was blocked by H_1-receptor blocker, antazoline in a concentration-related manner. Antazoline shifted the concentration response curve of histamine to the right in a parallel manner and there was no block of maximal responses. These observations suggested competitive antagonism. Furthermore, the slope of pA plot was not significantly different from the theoretical value of unity for competitive antagonist (Arunlakshana and Schild, 1959). The pA_2 value of antazoline with histamine was 8.84. This value is about one log unit higher than those reported for this H_1-receptor blocker by Reuse (1948) with guinea-pig ileum (pA_2 = 7.55) and by Gulati et al., (1968) with posterior vena cava strip ofrabbit (pA_2 = 7.37). Thus antazoline seems to possess higher affinity for H_1 receptors of this tissue than for those of other tissues.

5.2. Trachea

ISO caused concentration-related relaxation of goat trachea contracted with pilocarpine or KCl but had no effect on the trachea per se. Thus the goat trachea like the rat trachea does not seem to have intrinsic tone (Burn and Doe, 1978). Propranolol blocked the relaxant effect of ISO in a concentration-

related manner and shifted the concentration-response curve of ISO to the right in a parallel manner and there was no block of maximal responses. These observations suggested competitive antagonism. Moreover, the slope of PA plot was not significantly different from the theoretical value of unity for competitive antagonism (Arunlakshana and Schild, 1959). The pA_2 value of propranolol was 7.78 and is close to the pA_2 values for this beta-blocker reported by Takagi and Takayanagi (1970) with guinea-pig tracheal muscle (pA_2 = 7.2), by Gulati et al. (1973) with rabbit aortic strip (pA_2 = 7.05), by Ariens (1967) with calf tracheal muscle (pA_2 = 8.0) and by Wesserman and Bernard (1974) with dog heart (pA_2 = 7.26). Thus, the beta-receptors of this tissue seem to be similar to those of other tissues.

Relaxant responses to ISO were not modified by phentolamine. Rajani et al. (1977) and Chand et al. (1979) observed potentiation of relaxant action of ISO by phenoxybenzamine on guinea-pig trachea and goat trachea respectively. To explain this potentiation, Rajani et al. (1977) suggested inhibition of modulatory presynaptic alpha-receptor by phenoxybenzamine causing more release of NA. Chand et al., (1979) suggested the presence of scanty postsynaptic alpha-receptors (mediating contractions), the block of which by phenoxybenzamine accounted for potentiation. No modification of ISO responses by phenoxybenzamine was observed in the present study and it is, therefore, suggested that both presynaptic and postsynaptic alpha-receptors may be absent in goat trachea.

Relaxant responses to ISO were potentiated by normetanephrine. Since normetanephrine is a prototype $uptake_2$ blocker (Iversen, 1965b), it is quite likely that potentiation is due to this mechanism. The generalized inhibitory action of lower temperature on enzyme activity may account for the enhanced relaxant activity of ISO at low bath temperature.

Histamine-induced contractions of goat trachea were antagonized by mepyramine (a selective H_1-receptor antagonist) but not by metiamide. Similarly, H_1 histamine receptor-mediated contractions of trachea of horse, sheep and pig have been documented in the literature (Eyre, 1969; Chand and Eyre, 1977; Chand and DeRoth, 1978). Nagchaudhuri and Lahiri (1974) and Chand et al. (1979) also described H_1-receptor mediated contractions of goat trachea. However, Chand et al. (1979) also reported a scanty population of 'inhibitory' H_2-receptors in goat trachea. The present results indicated the absence of H_2-receptor in goat trachea since histamine failed to produce any relaxation of this tissue contracted with KCl.