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# Adrenergic responses of the rabbit stomach serosal strip and their modification by monoamine oxidase inhibitors and anti-adrenergic drugs

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The rabbit stomach serosal strip, was found to contract to adrenaline and noradrenaline but not to isoprenaline. The contractile response could be totally abolished by phenoxybenzamine but was not influenced by propranolol, indicating that the preparation has almost exclusively α-adrenoceptors. The responses to adrenaline and noradrenaline were markedly potentiated in the presence of monoamine oxidase inhibitors, guanethidine or reserpine, indicating the presence of MAO activity in the tissue and possibly catecholamine stores. The functional state of the latter has not been conclusively established, since tyramine, an indirectly acting amine, was unable to elicit a response qualitatively similar to that of adrenaline, even in the presence of nialamide or tranylcypromine.

It was previously shown by Khayyal, Tolba & others (1974) that the rabbit stomach serosal strip was especially sensitive to acetylcholine and could be used for its assay and that the preparation was stimulated by adrenaline and noradrenaline. We have examined further the effect of the sympathomimetic amines on the preparation and the modulatory effects of nialamide, tranylcypromine, reserpine and guanethidine, which are drugs known to modify sympathetic responses.

## MATERIALS AND METHODS

The rabbit stomach serosal strip (Khayyal & others 1974) was suspended in oxygenated Tyrode solution at 37°. The effect of adrenaline hydrogen tartrate and noradrenaline bitartrate (1 $\mu$g ml$^{-1}$ bathing fluid) and isoprenaline sulphate and tyramine hydrochloride (40 and 50 $\mu$g ml$^{-1}$ bathing fluid respectively) was studied. The drugs were left in contact with the preparation for 1 min.

Before testing the action of the sympathomimetic amines again, nialamide hydrochloride or tranylcypromine (30 $\mu$g ml$^{-1}$) or guanethidine sulphate (5 $\mu$g ml$^{-1}$) or reserpine (2·5 $\mu$g ml$^{-1}$) in the bathing fluid were allowed in contact for 30 min.

Serosal strips from animals chronically treated with nialamide hydrochloride or tranylcypromine sulphate, (2 mg kg$^{-1}$) guanethidine sulphate (10 mg kg$^{-1}$) or reserpine (0·1 mg kg$^{-1}$), all being given intramuscularly for 10 days, were prepared from rabbits killed on the eleventh day and the preparations subjected to the action of the sympathomimetic amines added in the same concentrations as before.

## RESULTS

### *Effect of sympathomimetics alone*

The addition of either adrenaline or noradrenaline (1 $\mu$g ml$^{-1}$ bathing fluid) produced a contractile response of the serosal strip. This response was not affected by the β-adrenoceptor blocker, propranolol (1 $\mu$g ml$^{-1}$). However, the α-adrenoceptor blocker, phenoxybenzamine (50 ng ml$^{-1}$), reduced the response to both adrenaline and noradrenaline and at higher concentrations abolished the catecholamine responses. Neither isoprenaline nor tyramine induced any visible response.

Table 1. *The mean responses af the rabbit stomach serosal strip to adrenaline or noradrenaline (1μg ml$^{-1}$ after treatment with nialamide, tranylcypromine, guanethidine or reserpine, when added to the bath (1) or after chronic treatment for 10 days before the experiment (2).* The figures are means ± s.e.; n = 5.

| | Responses to Adrenaline | | | Responses to Noradrenaline | | |
|---|---|---|---|---|---|---|
| Drug | Control | 1 | 2 | Control | 1 | 2 |
| Nialamide | 7·4 (±0·41) | 9·9 (±0·45) | 11·6 (±0·98) | 1·4 (±0·13) | 3·5 (±0·23) | 6·5 (±0·65) |
| Tranylcypromine | 3·2 (±0·22) | 9·3 (±0·21) | 10·4 (±0·21 | 1·6 (±0·14) | 5·2 (±0·16) | 6·7 (±0·21) |
| Guanethidine | 3·8 (±0·20) | 7·0 (±0·69) | 1·0 (±0·03) | 4·0 (±0·24) | 6·1 (±0·47) | 1·16(±0·02) |
| Reserpine | 4·5 (±0·43) | 9·0 (±0·27) | 8·2 (±0·22) | 2·6 (±0·22) | 7·0 (±0·59) | 5·8 (±0·13) |

† Correspondence.

*Effect of nialamide*

Nialamide in contact with the strip for 30 min, potentiated the responses to both adrenaline (Fig. 1) and noradrenaline while not affecting the responsiveness to isoprenaline or tyramine.

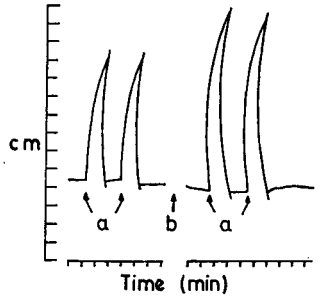


FIG. 1. Typical responses of the rabbit stomach serosal strip to adrenaline (a). Nialamide (30 $\mu$g ml$^{-1}$) added to the bath for 30 min (b) markedly enhanced the contractions to adrenaline (1 $\mu$g ml$^{-1}$).

The effect of chronic treatment with nialamide enhanced the response to adrenaline and noradrenaline to nearly twice that in acute experiments (Tables 1 and 2).

*Effect of tranylcypromine*

Tranylcypromine also significantly potentiated the response of the serosal strip to adrenaline and noradrenaline, both in acute and in chronic experiments, the effect on the responses to adrenaline being much more marked (Tables 1 and 2).

Table 2. *The mean ($n = 5$) percentage increase (positive values) or decrease (negative values) in response of the rabbit stomach serosal strip to adrenaline and noradrenaline (1 $\mu$g ml$^{-1}$) after treatment with nialamide, tranylcypromine, guanethidine or reserpine when added to the bath (1) or after treatment of the animals for 10 days before the experiment (2).*

| | % Change in adrenaline response | | % Change in noradrenaline response | |
|---|---|---|---|---|
| Drug | 1 | 2 | 1 | 2 |
| Nialamide | + 32* | + 57* | +150*** | +374*** |
| Tranylcypromine | +196*** | +225*** | +225*** | +318*** |
| Guanethidine | + 84** | − 73*** | + 52** | − 60*** |
| Reserpine | +100*** | + 82*** | +169*** | +128*** |

*$P < 0.05$, **$P < 0.025$, ***$P < 0.005$. As measured by—Student's $t$-test applied to the results of drug treatment compared to control responses to adrenaline and noradrenaline.

*Effect of guanethidine*

Guanethidine significantly potentiated the contractions of the serosal strip to both adrenaline and noradrenaline. Strips from rabbits chronically treated with guanethidine showed a markedly reduced response to the two amines (Tables 1 and 2). The drug did not alter the responses to isoprenaline or tyramine.

*Effect of reserpine*

After acute and chronic reserpine treatment of the strips the responses to adrenaline and noradrenaline were markedly enhanced (acute see Fig. 2).

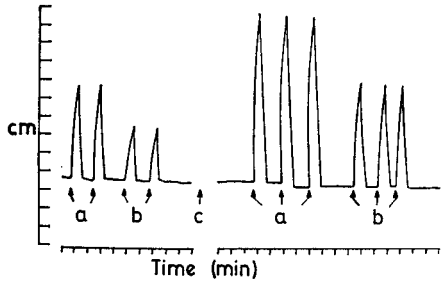


FIG. 2. The comparative effect of (a) adrenaline and (b) noradrenaline (1 $\mu$g ml$^{-1}$) on the rabbit stomach serosal strip before and after the addition of reserpine to the bath for 30 min (c) at a dose of 2·5 $\mu$g ml$^{-1}$. Reserpine significantly potentiated the responses to both catecholamines.

## DISCUSSION

The rabbit stomach serosal strip contracts to adrenaline and noradrenaline, but not to isoprenaline or tyramine. The response to the naturally occurring catecholamines was inhibited by phenoxybenzamine, but not by propranolol. Thus it would seem that the preparation has predominantly, if not exclusively, $\alpha$-adrenoceptors. However, unlike the $\beta$-adrenoceptors elsewhere in the gastrointestinal tract, which are predominantly inhibitory, those in the strip preparation are stimulated by adrenaline and noradrenaline. In this way, the preparation apparently behaves in a similar manner to the muscularis mucosa of the oesophagus of the pig (Burnstock, 1960) and guinea-pig (Bailey, 1965) upon which adrenaline was shown to produce depolarization and contraction. In the dog stomach too, Ichikawa & Bozler (1955) have shown that adrenaline, after producing an initial stage of inhibition associated with hyperpolarization, caused a secondary stage of excitement associated with depolarization. The response of the guinea-pig serosal strip to catecholamines is similar to that reported for the rabbit preparation (Khayyal, unpublished observations) while on the rat fundus strip (Vane, 1957) adrenaline and noradrenaline are inhibitory (Armitage & Vane, 1964). When whole muscle strips (including both

serosal and mucosal layers) of rabbit were used the excitatory responses to the catecholamines were markedly reduced (unpublished observations). This reduction in sensitivity is non-specific, since it was also observed with acetylcholine, indicating perhaps that the anatomical structure of the whole muscle strip, rather than the presence of $\beta$-adrenoceptors, has a role in modifying the contractile response.

Both nialamide and tranylcypromine, MAO inhibitors of the hydrazine and non-hydrazine types respectively, either acutely or chronically potentiated the responses to adrenaline and noradrenaline 2–3 times (Table 2) indicating the presence of MAO activity in the preparation. Vane (1959) found MAO activity in the rat fundus strip.

Davey, Farmer & Reinert (1963), Furchgott & Garcia (1968) and Iversen & Callingham (1971) considered that some MAO inhibitors interfered with uptake$_1$ of catecholamines, leading to an increase in their concentration at the receptor sites (Trendelenburg, 1966). However, Kalsner & Nickerson (1969 a, b) and Kalsner (1975) showed that uptake$_1$ played only a minor role in inactivating high concentrations of noradrenaline, and that the major role was played by extraneuronal mechanisms (uptake$_2$), followed by deamination and *O*-methylation. Our present findings appear to be more in agreement with Kalsner's findings since inhibition of deamination by either nialamide or tranylcypromine would block a major pathway of catecholamine inactivation, leading to the potentiation of the responses observed. Tranylcypromine, being a more potent inhibitor of MAO than nialamide (Pletscher, 1966), had a greater potentiating effect on the responses of the strip to catecholamines.

The *in vitro* potentiation of the responses to adrenaline and noradrenaline of guanethidine could be explained on the basis of interference with neuronal uptake of catecholamines (Stafford, 1963; Iversen, 1965; Trendelenburg, 1966). Chronic pretreatment with guanethidine, however, led to a reduction in sensitivity of the serosal strip and to adrenaline and noradrenaline. Such an effect may be due to a non-specific direct depressant action of guanethidine on the smooth muscle cells of the serosal layer. In fact, the response of the strip to acetylcholine was also reduced after guanethidine (unpublished observation). Dzoljic (1964) and Dzoljic, Van Noordwijk & others (1964) demonstrated similar effects of guanethidine (15 mg kg$^{-1}$) on the response of the rabbit ileum and rat stomach to the stimulant action of histamine and barium chloride. The dose was comparable to the dose we used.

The subsensitivity of the preparation after chronic guanethidine treatment may also be explained on the basis of receptor deformation, a view supported by the findings of Jensen-Holm (1967), Jensen-Holm & Juul (1971) in the rat superior cervical ganglion.

Trendelenburg (1963) showed that chronic pretreatment with reserpine could cause supersensitivity to catecholamines resembling that observed after chronic preganglionic denervation. This 'decentralization' supersensitivity is non-specific and not related to the noradrenaline content of the sensitized tissue. Short term pretreatment, however, caused depletion of noradrenaline stores but no supersensitivity. In our acute experiments, the addition of reserpine led to marked potentiation of the adrenaline and noradrenaline responses which could possibly have been due to summation of the effects of endogenous noradrenaline (increased in the proximity of the receptors, because of the releasing action of reserpine) to the action of the added amines (Trendelenburg, 1963) or due to changes in tissue calcium utilization (Hudgins & Harris, 1970). The supersensitivity to catecholamines after reserpine is not likely to be due to inhibition of neuronal uptake of noradrenaline since there is evidence that reserpine does not reduce neuronal uptake (Iversen, Glowinski, Axelrod, 1966; Haggendal & Malmfors, 1969).

It would thus seem that the rabbit stomach serosal strip contains $\alpha$-adrenoceptor excitatory receptors, whose sensitivity to adrenaline and noradrenaline can be significantly enhanced with MAO inhibitors, guanethidine or reserpine. MAO activity in the tissue is thus very probable, but not the presence of intact functioning tissue amine stores, since tyramine failed to elicit a response. The effects of guanethidine and reserpine may be explained independently from intact amine stores.

## REFERENCES

ARMITAGE, A. K. & VANE, J. R. (1964). *Br. J. Pharmac. Chemother.*, **22**, 204–210.
BAILEY, D. M. (1965). *J. Pharm. Pharmac.*, **17**, 782–787.
BURNSTOCK, G. (1960). *Nature (Lond.)*, **186**, 727–728.
DAVEY, M. J., FARMER, J. B. & REINERT, H. (1963). *Br. J. Pharmac. Chemother.*, **20**, 121–134.
DZOLJIC, M. (1964). *Archs Int. Pharmacodyn. Thér.*, **151**, 334–342.

DZOLJIC, M., VAN NOORDWIJK, J., AARSEN, P. N. & LOEBIS, L. H. (1964). *Ibid.*, **151**, 343–351.
FURCHGOTT, R. F. & GARCIA, P. S. (1968). *J. Pharmac. exp. Ther*, **163**, 98–122.
HAGGENDAL, J. & MALMFORS, T. (1969). *Acta physiol. scand.*, **75**, 33–38.
HUDGINS, P. M. & HARRIS, T. M. (1970). *J. Pharmac. exp. Ther.*, **175**, 609–618.
ICHIKAWA, S. & BOZLER, E. (1955). *Am. J. Physiol.*, **182**, 92–96.
IVERSEN, L. L. (1965). In: *Advances in Drug Research*, vol. 2, pp. 1–46. Editors: Harper, N. J. and Simmonds, A. B., London: Academic Press.
IVERSEN, L. L., GLOWINSKI, J. & AXELROD, J. (1966). *J. Pharmac. exp. Ther.*, **150**, 173–183.
IVERSEN, L. L. & CALLINGHAM, B. A. (1971). *Adrenergic Transmission.* In: *Fundamentals of Biochemical Pharmacology.* Editor: Bacq, Z. M., Oxford and New York: Pergamon.
JENSEN-HOLM, J. (1967). *Acta pharmac. tox.*, **25**, *Suppl.* 4, 74.
JENSEN-HOLM, J. & JUUL, P. (1971). *Ibid.*, **30**, 308–320.
KALSNER, S. & NICKERSON, M. (1969a). *J. Pharmac. exp. Ther.*, **165**, 152–165.
KALSNER, S. & NICKERSON, M. (1969b). *Br. J. Pharmac.*, **35**, 440–455.
KALSNER, S. (1975). *Ibid.*, **53**, 267–277.
KHAYYAL, M. T., TOLBA, H. M., EL-HAWARY, M. B. & ABDEL-WAHED, S. (1974). *Eur. J. Pharmac.*, **25**, 281–290.
PLETSCHER, A. (1966). *Pharmac. Rev.*, **18** (1), 121–129.
STAFFORD, A. (1963). *Br. J. Pharmac. Chemother.*, **21**, 361–367.
TRENDELENBURG, U. (1963). *Pharmac. Rev.*, **15**, 225–276.
TRENDELENBURG, U. (1966). *Ibid.*, **18**, 629–640.
VANE, J. R. (1957). *Br. J. Pharmac. Chemother.*, **12**, 344–349.
VANE, J. R. (1959). *Ibid.*, **14**, 87–98.