Cardiovascular responses to amantadine hydrochloride in the rat and rabbit

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Amantadine, an antiviral agent, has been reported to be effective in the treatment of Parkinson's disease (Schwab, England, Poskanzer & Young, 1969). It has been suggested that amantadine releases catecholamines from nerve storage sites on the basis of a pressor response in the anaesthetized dog which was increased by prior injection of dopamine (DA) (Grelak, Clark, Stump & Vernier, 1970). We have investigated the cardiovascular actions of amantadine and attempted to confirm an interaction with dopamine.

In the urethane anaesthetized rat and rabbit amantadine (20 μg to 2 mg) produced a pressor response with no change in heart rate. DA had qualitatively different effects and was ten times more potent. In the rat the DA pressor effect was accompanied by an increase in heart rate and in the rabbit it gave a depressor response. No potentiation of amantadine was observed in the rat, after injection of DA.

Reserpine pretreatment (5 mg/kg, I.P., 18 h) and guanethidine, in a dose which blocked the pressor response to tyramine (2 μg), did not significantly affect the amantadine pressor response in the rat. Repeated injections af amantadine showed no evidence of tachyphylaxis and there was no cross tachyphylaxis with tyramine. In the pithed rat the mean blood pressure responses to low doses of amantadine (20 to 200 μg) were reduced but the response to 2 mg was increased.

Phentolamine in a dose which antagonized the pressor response to noradrenaline (100 ng) also blocked the pressor response to amantadine. However, when amantadine was tested on the rat isolated aortic strip it produced a maximum contraction at 62·5 μg/ml, which was only 15% of a noradrenaline maximum. Addition of amantadine relaxed an aortic strip fully contracted with noradrenaline.

In conclusion, amantadine is a weak pressor agent in the rat and rabbit and is neither DA-like nor tyramine-like in its actions. There is some evidence that an intact central nervous system is required for the pressor response to low doses, and work on the isolated aorta suggests it may be a partial agonist at α-adrenoceptors.

This work was supported by the M.R.C.

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Receptors for dopamine in some isolated vascular tissues of the dog

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In many peripheral tissues dopamine (DA) has been shown to have agonist activity at both α- and β-adrenoceptors (Rossum, 1965 and Tsai, Langer & Trendelenburg,

1967). However, recent evidence has suggested the presence of a receptor specific for DA in the renal vasculature of the anaesthetized dog (Goldberg & Yeh, 1971). In the present work *in vitro* techniques have been used to investigate this mechanism further.

Segments of abdominal aorta, posterior vena cava, renal artery and vein were taken from mongrel dogs of either sex, weighing from 10 to 17 kg, killed by electrocution. Helically cut strips were mounted vertically within a tissue bath containing Krebs solution, maintained at 37° C and bubbled with 5% carbon dioxide in oxygen. Tension changes were measured isometrically. Both agonists and antagonists were administered cumulatively and 30 min was allowed for equilibration of each agonist.

Concentration-effect curves for the tension increases produced by adrenaline, noradrenaline, phenylephrine and N-methyldopamine were constructed for all of the tissues and the EC_{50} values compared with those for dopamine. Potency ratios for DA (with noradrenaline defined at 1·00) were 0·02, 0·03, 0·03 and 0·02 for abdominal aorta, renal artery, posterior vena cava and renal vein respectively (number of determinations 15–17). The order of potency of these compounds on each tissue was the same and consistent with the responses being mediated by α-adrenoceptors.

The possibility that, in renal tissues, 'DA receptors' made up a significant portion of the total receptor population was tested. pA_4 values for phentolamine and thymoxamine, α-adrenoceptor antagonists (Birmingham, Ernest & Newcombe, 1969 and Rossum, 1965), and haloperidol and pimozide, compounds thought to have 'DA receptor' blocking actions (Janssen, Niemegeers, Schellekens, Dresse, Lenaerts, Pinchard, Schaper, Van Nueten & Verbruggen, 1968) were compared using phenylephrine and DA as agonists. Neither haloperidol nor pimozide showed selectivity towards the response to DA in any of the tissues. The pA_4 values with phenylephrine as agonist were $7{\cdot}66 \pm 0{\cdot}06$, $7{\cdot}00 \pm 0{\cdot}10$, $7{\cdot}41 \pm 0{\cdot}04$ and $7{\cdot}06 \pm 0{\cdot}04$ for haloperidol, pimozide, phentolamine and thymoxamine respectively. The corresponding figures with DA as agonist were $7{\cdot}61 \pm 0{\cdot}05$, $7{\cdot}00 \pm 0{\cdot}12$, $7{\cdot}38 \pm 0{\cdot}05$ and $7{\cdot}08 \pm 0{\cdot}07$ respectively (means $\pm$ standard errors, pooled from 2–4 determinations on each tissue). In each case there were no significant differences between values for phenylephrine and DA ($P > 0{\cdot}05$, Student's t test, 2 tailed).

Tsai, Langer & Trendelenburg (1967) have shown DA to possess both β-adrenoceptor stimulating properties and indirect actions. The possibility that these factors may have modified the pA_4 values was tested using propranolol (4·75 μM), cocaine (10 μM), guanethidine (10 μM) and desmethylimipramine (10 nM). On the renal artery, propranolol did not affect the pA_4 value for phentolamine using either phenylephrine or DA as agonist. While cocaine, guanethidine and desmethylimipramine left responses to DA unchanged on preparations in which noradrenaline's response was potentiated and tyramine's (100 μM) abolished.

These experiments demonstrate that the receptors mediating tension changes to DA are identical to those mediating similar responses to phenylephrine and are likely to be α-adrenoceptors. The results therefore suggest that the locus of the 'DA receptor' *in vivo* (Goldberg & Yeh, 1971) is not the renal artery or vein.

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Ocular irritation tests

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Investigations on the potential of substances to cause damage to the eye cover numerous products used in industry, agriculture and medicine. The more important considerations in ocular irritation tests are discussed.

Observations are required on both formulations and active ingredient. The latter is tested as undiluted liquid or solid, and at serial dilutions to determine the maximum concentration having no undesirable effects. Groups of 6 or more animals are required. The rabbit is frequently used, but where possible the response should be checked against primates (Beckley, Russell & Rubin, 1969).

Two important factors are the solvent and the volume instilled (Ballantyne, Gazzard & Swanston, 1972). Non-irritant solvents without effects on intraocular tension are required; saline, polyethylene glycol 300, propylene glycol and glyceryl triacetate are examples. A convenient volume is 0·1 ml.

Eyes are examined and photographed at 10 min, 1, 6 and 24 h, and thereafter daily for 2 weeks. Macroscopic observations should be supplemented with biomicroscopy and fundoscopy. Cumulative single figure scoring systems, like that of Draize (1959), involve calculating a single average of all effects observed, and incorporate a heavy bias on corneal pathology. Such scores are uninformative about responses of individual tissues and thus, a specific Draize score could result from two entirely different reactions. Scoring bias ignores the significance of some effects; for example, severe contracture of the eyelids may be equally as serious as keratitis. In our laboratories means for each effect, scored on a 5 point scale, are calculated for the group at each inspection period. Such mean scores, recorded as a function of time on tables or graphs, are easier to interpret and more meaningful than cumulative weighted scores. Thus, they readily allow comparison of the effects of varying concentrations (Fig. 1) and facilitate comparison of particular effects produced by different oculotoxic drugs.

Macroscopic observations should be supplemented with terminal and sequential histopathology. Any advantages of the cup-aspirator technique (Buehler & Newmann, 1964) require confirmation, but applanation tonometry may be an additional valuable technique in ophthalmic toxicology (Ballantyne, Gazzard & Swanston, 1972).