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Impairment of sympathetic nerve responses by dopa, dopamine and their α-methyl analogues

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Dopa and dopamine reduced the response of the nictitating membrane to post-ganglionic sympathetic nerve stimulation in cats pretreated with the monoamine oxidase inhibitor pargyline. A similar reduction could be obtained with α-methyldopa and α-methyldopamine in untreated cats. The sensitivity of the membrane to injected noradrenaline, α-methylnoradrenaline or adrenaline was not reduced. The possible mechanism of the anti-hypertensive action of monoamine oxidase inhibitors and α-methyldopa is discussed in the light of these findings.

THE impairment of the response to sympathetic nerve stimulation by indirectly-acting sympathomimetic amines has been described by several investigators (Aström, 1949; Day, 1962; Day & Rand, 1963a,b). Those amines which are substrates for monoamine oxidase are only effective in the presence of a monoamine oxidase inhibitor (Day & Rand, 1963a,b), but amines possessing an α-methyl substituent, which confers immunity to attack by monoamine oxidase (Blaschko, Richter & Schlossmann, 1937) impair sympathetic responses in untreated animals.

Dopamine, the immediate precursor of noradrenaline, has direct sympathomimetic actions, although in some tissues its action is partly direct and partly indirect (Bejrablaya, Burn & Walker, 1958; Strömblad, 1960). Similarly, α-methyldopamine acts both directly and indirectly. Evidence for its indirect action is the marked depletion of tissue stores of noradrenaline it effects (Porter, Totaro & Leiby, 1961). Dopamine is a good substrate for monoamine oxidase (Blaschko & others, 1937) whilst α-methyldopamine is not metabolised by this enzyme (Carlsson & Lundqvist, 1962).

The purpose of this investigation was to determine whether dopamine and α-methyldopamine behaved like other indirectly-acting sympathomimetic amines in impairing responses to sympathetic nerve stimulation. The amino-acid precursors of these amines, dopa and α-methyldopa were also tested.

Experimental

METHODS

Cats, weighing 2–3·5 kg, were used. Anaesthesia was induced with halothane in nitrous oxide and oxygen (3:1 v/v) and maintained by intravenous injection of 80 mg/kg chloralose. In some experiments additional injections of pentobarbitone were necessary since infusions of dopamine, and more particularly dopa, lightened anaesthesia in animals pretreated with the monoamine oxidase inhibitor. Injections and infusions were made into the right femoral vein. The infusions of dopa, dopamine, α-methyldopa and α-methyldopamine were made at a constant rate for a period of 50 min. The amounts of these substances referred to

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are the total amounts in mg/kg given within this period. The contractions of the right nictitating membrane were recorded on smoked kymograph paper with an isotonic frontal writing lever of 8 g tension and 15 times magnification. The postganglionic cervical sympathetic nerves were exposed on the right side by partial removal of the trachea and oesophagus; the trachea was cannulated low in the neck. The postganglionic sympathetic nerves were laid over bipolar platinum electrodes and the superior cervical ganglion was crushed. The edges of the wound were stitched to a metal frame and the dissected tissue flooded with liquid paraffin. The nerve was stimulated with rectangular wave pulses from an electronic stimulator. The pulses were of 1 msec duration, 10 V strength, and were given at 1, 5 or 10 pulses/sec. They were applied for 20 sec, which allowed the membrane to reach the full response at each of the frequencies applied. In each experiment the following procedure was observed: injections of noradrenaline, adrenaline, or α-methylnoradrenaline were given intravenously in amounts which produced a well defined contraction of the membrane followed by three periods of nerve stimulation. This sequence was performed in repeated cycles before and after the infusions. The monamine oxidase inhibitor employed in this study was pargyline (Taylor, Wykes, Gladish & Martin, 1960). Cats were pretreated with pargyline, which was administered subcutaneously as a single dose (25–50 mg/kg), or as four divided doses (total 100 mg/kg) during the two days preceding the experiment. Experiments were started 16 hr after the last injection. The decarboxylase inhibitor NSD 1055 (Hansson & Clark, 1962) was given intravenously.

DRUGS

The following drugs were used: (—)-adrenaline acid tartrate, (—)-noradrenaline acid tartrate, L-dopa, dopamine hydrochloride, 4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate (NSD 1055), L-α-methyldopa, (—)-α-methylnoradrenaline, (±)-α-methyldopamine, pargyline hydrochloride (*N*-benzyl-*N*-methyl-2-propynylamine). Doses were given in terms of these compounds.

Results

There was no difference between control cats and cats pretreated with pargyline in the sensitivity of the membrane to injected noradrenaline, adrenaline and nerve stimulation. The response of the membrane to nerve stimulation and injected noradrenaline and adrenaline was determined for up to 4 hr after the end of the infusions. Within this period, deterioration of the preparation occurred in some experiments, the response to the highest frequency of nerve stimulation being most affected. This effect could be clearly differentiated from the impairment produced by the infusions.

The infusion of dopa at 10 mg/kg and above, produced a sustained contraction of the membrane; this effect was more prolonged and was produced by smaller amounts of dopa in cats pretreated with pargyline. α-Methyldopa, 100 and 200 mg/kg, did not cause the membrane to

contract. Dopamine, 5 and 10 mg/kg, and α-methyldopamine, 10 and 20 mg/kg, caused a prolonged contraction of the membrane; the effect of dopamine, as for dopa, was more prominent in cats pretreated with pargyline. The contraction persisted for up to 1½ hr after termination of the infusions; the responsiveness of the membrane to nerve stimulation or to intravenous injections was not determined until the contraction produced by the infusion had subsided. The administration of the decarboxylase inhibitor NSD 1055, 20 mg/kg, before the infusion of dopa, 10 mg/kg, prevented the appearance of the prolonged contraction. However, a subsequent infusion of dopamine produced a prolonged contraction. It is perhaps of interest that infusions of dopa and dopamine resulted in copious salivation in all cats, particularly those pretreated with pargyline. NSD 1055 prevented this response to dopa but not to dopamine.

EFFECT OF INFUSIONS ON THE RESPONSE OF THE MEMBRANE TO POSTGANGLIONIC NERVE STIMULATION

In three experiments the infusion of dopa, 5, 10 and 20 mg/kg, did not cause impairment of the response to nerve stimulation. In a further two experiments in cats pretreated with pargyline, dopa, 5 and 10 mg/kg, reduced the response to nerve stimulation (Fig. 1A). However, when an injection of NSD 1055 was given before the infusion there was only a slight reduction in the responses (Fig. 1B).

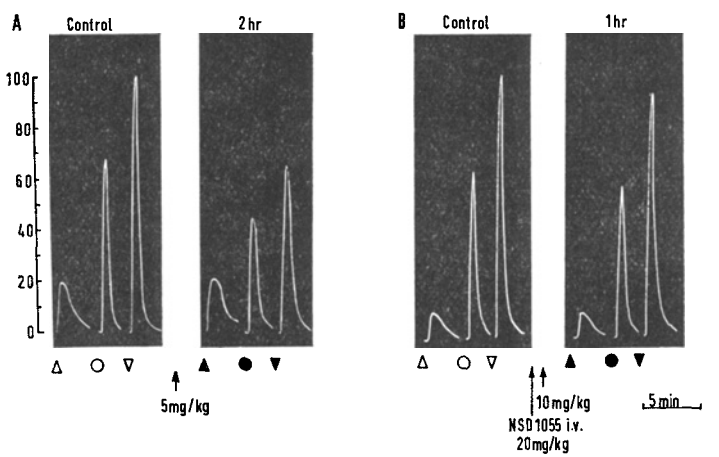

FIG. 1. Infusion of dopa, 5 and 10 mg/kg in 50 min, in two cats pretreated with pargyline. Effect on the response of the cat nictitating membrane to injected noradrenaline (△) and postganglionic nerve stimulation (1 pulse/sec ○; 5 pulses/sec ▽). Infusion made at ↑. In B infusion preceded by injection of 20 mg/kg NSD 1055. Open symbols before, closed symbols at stated time after termination of infusion.

The infusion of dopamine, 5 mg/kg in one experiment and 10 mg/kg in two experiments, did not result in any marked change in the responses to nerve stimulation. In one experiment there was a gradual decline in the responses while in two experiments they were unchanged or increased. In three experiments in cats treated with pargyline, dopamine, 5 mg/kg in

one experiment and 10 mg/kg in two experiments, caused impairment of responses to nerve stimulation (Fig. 2A). In the experiment with 5 mg/kg of dopamine, there was some recovery of the responses within 3 hr. The

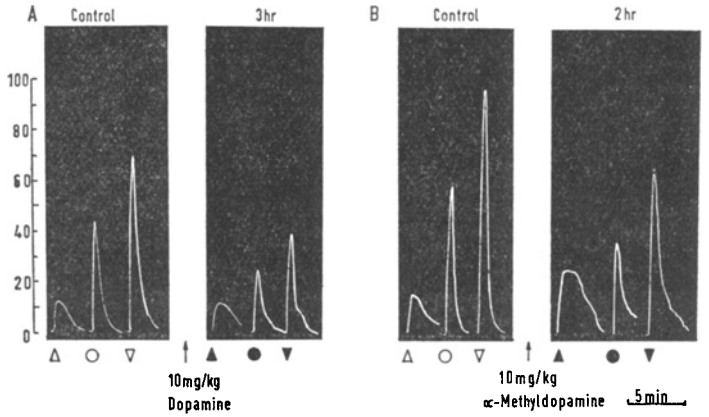

FIG. 2. Infusion of dopamine 10 mg/kg in 50 min in cat pretreated with pargyline (A) and α-methyldopamine 10 mg/kg in 50 min in untreated cat (B). Effect on the response of the cat nictitating membrane to, in A, injected noradrenaline (△) or, in B, α-methylnoradrenaline (△) and postganglionic nerve stimulation (1 pulse/sec ○; 5 pulses/sec ▽). Open symbols before, closed symbols at stated time after termination of infusion.

responses were also impaired in the cat given NSD 1055 before the infusion of dopamine.

In two experiments, infusions of α-methyldopa, 100 and 200 mg/kg, were given. No permanent effect on nerve stimulation was observed with the lower dose; there was some depression immediately after termination of the infusion but the responses then recovered fully. The infusion of 200 mg/kg of α-methyldopa produced a gradually developing impairment of the responses.

In two experiments, α-methyldopamine 10 and 20 mg/kg, caused impairment of the responses to nerve stimulation (Fig. 2B).

EFFECT OF INFUSIONS ON RESPONSE OF THE MEMBRANE TO INJECTED CATECHOLAMINES

After dopa, the responses to noradrenaline and adrenaline were enhanced in normal cats but not in those pretreated with pargyline. After dopamine the responses to noradrenaline and adrenaline were unchanged, either in the presence or absence of pargyline. α-Methyldopa and α-methyldopamine enhanced the response of the membrane to noradrenaline and α-methylnoradrenaline. α-Methylnoradrenaline was included in the experiments in which α-methyldopa and α-methyldopamine were investigated since an approximate estimate of the potency relative to noradrenaline was required. Neither noradrenaline nor α-methylnoradrenaline were examined for chemical purity, but the activity of α-methylnoradrenaline on the membrane was approximately half that of noradrenaline, which is in agreement with Ahlquist (1948).

Discussion

In the presence of a monoamine oxidase inhibitor the response of the nictitating membrane to postganglionic nerve stimulation was impaired after infusion of dopa and dopamine, although the response to injected noradrenaline and adrenaline remained unchanged or enhanced. Such impairment did not occur in normal cats. α-Methyldopa and α-methyldopamine impaired the response to nerve stimulation in normal animals and enhanced the response to injected noradrenaline and α-methylnoradrenaline. A gradually developing impairment observed after α-methyldopa has been reported previously by Day & Rand (1964). This may be explained by the very slow rate of decarboxylation of this substance in tissues (Lovenburg, Weissbach & Udenfriend, 1962). In contrast, dopa in the presence of pargyline produced an immediate impairment in much smaller amounts than those required for α-methyldopa. However dopa did not impair the responses after injection of a decarboxylase inhibitor (NSD 1055) although NSD 1055 did not affect the impairment produced by dopamine. The dose of NSD 1055 employed here prevented the vasopressor action of dopa (Horlington, M., personal communication); this is indicative of the inhibition of decarboxylase activity (Clark, 1959). Similarly, the administration of a decarboxylase inhibitor prevented the hypotensive and catecholamine-depleting action of α-methyldopa (Davis, Drain, Horlington, Lazare & Urbanska, 1963).

The results suggest that α-methyldopa or dopa in the presence of pargyline exert their effects after decarboxylation, and that the metabolites are the active substances. Now α-methyldopa undergoes decarboxylation and subsequent hydroxylation *in vivo* (Carlsson & Lundqvist, 1962) yielding first α-methyldopamine and then α-methylnoradrenaline. α-Methylnoradrenaline has less activity than noradrenaline and could therefore cause impaired responses by acting as a false transmitter (Day & Rand, 1963c, 1964). The impairment of nerve responses produced by α-methyldopamine was approximately 40%, particularly at low stimulation rates, and since α-methylnoradrenaline has one half the potency of noradrenaline on the nictitating membrane of the cat (Ahlquist, 1948), this degree of impairment would require almost total replacement of the noradrenaline store with α-methylnoradrenaline. Observations of the effect of α-methyldopamine on noradrenaline levels in tissues indicate that this amount of depletion does not occur (Porter & others, 1961; Levine & Sjoerdsma, 1964). It must also be taken into consideration that the infusion of α-methyldopamine increased the sensitivity of the membrane to injected α-methylnoradrenaline. It seems unlikely that the formation and utilisation of α-methylnoradrenaline could account completely for the effects of α-methyldopa or α-methyldopamine. Dopa undergoes decarboxylation to form dopamine and is then hydroxylated to form noradrenaline (Blaschko, 1939). The formation of a false transmitter cannot account for the impairment observed in these experiments after infusion of dopa and dopamine. α-Methyldopamine, and dopamine in the presence of pargyline, produced similar degrees of impairment of

the responses and were active in similar doses. Therefore dopamine formed from dopa may accumulate after inhibition of monoamine oxidase, and α-methyldopamine formed from α-methyldopa may be responsible for the effects of the amino-acids. There are grounds for the hypothesis that accumulation of dopamine may account for the anti-hypertensive action of monoamine oxidase inhibitors. In animals treated with monoamine oxidase inhibitors, an increased dopamine content in peripheral tissues and an increased ability to hold injected dopamine has been demonstrated (Harrison, Levitt & Udenfriend, 1963). Extracts of sympathetic nerves incubated with dopa and the monoamine oxidase inhibitor, marsilid, synthesise increased amounts of dopamine (Goodall & Kirshner, 1958). A reduction in the amount of noradrenaline released by nerve stimulation from the isolated perfused spleen of cats pretreated with the monoamine oxidase inhibitor nialamide has been observed (Davey, Farmer & Reinert, 1963); the accumulation of dopamine may account for this finding. Other indirectly acting sympathomimetic amines, derived from naturally occurring amino-acids, which are substrates for amine oxidase, have been shown to produce impairment of sympathetic responses in the presence of a monoamine oxidase inhibitor (Day & Rand, 1963b). However, dopamine formed from dopa in the cytoplasm of adrenergic nerves is taken up by the noradrenaline store and converted to noradrenaline (Schumann, 1960). Excessive amounts of dopamine may be favourably sited to interfere or compete with either uptake or release of noradrenaline. The accumulation of dopamine would be favoured since conversion of the amino-acid to the amine is rapid, whilst hydroxylation of the amines proceeds more slowly (Hess, Connamacher, Ozaki & Udenfriend, 1961).

Similarly there are grounds for the hypothesis that the accumulation of α-methyldopamine may account for the anti-hypertensive action of α-methyldopa. α-Methyldopa is converted to α-methyldopamine and eventually to α-methylnoradrenaline; the presence of α-methyldopamine was detected for up to 24 hr in tissues of animals treated with α-methyldopa, whilst noradrenaline levels were decreased for considerably longer (Carlsson & Lundqvist, 1962). In man, large single doses of α-methyldopa lower blood pressure for approximately 24 hr (Dollery & Harrington, 1962). Therefore a similar mechanism may operate for α-methyldopamine as for dopamine, this substance accumulating because of the failure to be metabolised by monoamine oxidase. In this context, α-methyl-*m*-tyrosine is less efficacious than α-methyldopa in lowering blood pressure in man and animals (Stone, Porter, Watson & Ross, 1961; Horwitz & Sjoerdsma, 1963), whilst both substances produce similar degrees of depletion of tissue stores of noradrenaline in animals (Hess & others, 1961). α-Methyl-*m*-tyrosine is metabolised *in vivo* to α-methyl-*m*-tyramine and metaraminol (Carlsson & Lundqvist, 1962). Metaraminol, but not α-methyl-*m*-tyramine, is easily bound in tissues when injected intravenously (Alpers, Busfield & Shore, 1964) but both substances will deplete tissue stores of noradrenaline (Udenfriend & Zaltzman-Nirenberg, 1962). Now injected tyramine, unlike dopamine, is not easily bound by tissues, but is

rapidly converted to octopamine (Musacchio, Kopin & Snyder, 1964). When α-methyl-*m*-tyramine is generated *in vivo* from α-methyl-*m*-tyrosine, part will be lost from the tissue and excreted and part will be rapidly hydroxylated; thus very little of this substance may accumulate. It may be that α-methyl-*m*-tyrosine does not have marked anti-hypertensive actions since there is failure of the metabolite α-methyl-*m*-tyramine to accumulate in the vicinity of the noradrenaline store.

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