The effect of L-dopa on pupillary diameter in mice

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Summary. Injection of L-dopa in mice produces dose-dependent mydriasis. Pre-treatment with peripheral dopa-decarboxylase inhibitors (carbidopa and benserazide) or with an alpha-adrenergic blocking agent (phentolamine) abolishes the pupillary dilation caused by L-dopa. Pretreatment with fusaric acid, an inhibitor of dopamine-beta-hydroxylase, also antagonizes the mydriatic effect of L-dopa. Thus, our results suggest that the mydriasis produced in mice following the injection of L-dopa is caused by its peripheral conversion to noradrenaline, which stimulates alpha-adrenergic receptors in the dilator iridis. There was no evidence that stimulation of specific dopaminergic receptors was involved.

Ocular administration of dopamine and L-dopa induces pupillary dilation in humans. Spiers et al.[3] have suggested that L-dopa is rapidly converted to dopamine, which releases noradrenaline from sympathetic nerve endings in the iris. This effect is interesting because of the possible parallelism to other side-effects of L-dopa treatment[4].

The present study was aimed to establish and characterize further the pupillary effects of L-dopa. Our experiments were performed on mice, animals particularly suitable for this type of study. Pupillary measurements can be performed in this species rapidly and repeatedly without anesthesia; the weak light reflex of mice makes measurement accurate and independent of illumination[5].

Materials and methods. Animals: ICR male albino mice, weighing 20–35 g were used in all experiments.

Drugs: The following drugs were used: L-dopa, carbidopa and benserazide (Assia), phentolamine-methane-sulphonate (Ciba), fusaric acid (Sigma), and dopamine (Disco). L-dopa, carbidopa and benserazide were dissolved in buffer KCl-HCl, pH 1.5. Phentolamine and fusaric acid were dissolved in 0.9% NaCl solution. All drugs were injected i.p. Groups of 8–10 mice were used.

Pupillary diameter measurements: Pupillary diameter measurements and calculations of the results were done as described by Treister et al.[5]. Briefly, animals were held under a Zeiss model K 120/76 binocular microscope. One of the oculars was fitted with a divided 0.05-mm ruler. The total magnification was ×20. Both pupils were measured and the mean calculated.

Pupillary response to L-dopa: Groups of mice were injected with 100, 200 or 300 mg/kg L-dopa and pupillary measurements were performed. Control groups were injected with KCl-HCl buffer, pH 1.5, or with dopamine. Separate groups of mice were pretreated with dopa-decarboxylase inhibitors (benserazide or carbidopa 300 mg/kg) 1 h before the injection of 300 mg/kg L-dopa. Control groups received 300 mg/kg carbidopa or benserazide without L-dopa. Another group of mice was pretreated with 50 mg/kg fusaric acid 3 h before the injection of 300 mg/kg L-dopa. Phentolamine (50 mg/kg) was injected in a group of mice 30 min before the injection of 300 mg/kg L-dopa.

Results. L-dopa induces dose-dependent pupillary dilation in mice (fig. 1). The pupillary response develops rapidly, maximal response occurring 10 min after administration of the drug. Injection of the buffer KCl-HCl itself causes a slight (30%) pupillary dilation, the maximal effect occurring at 30 min. The dopa-decarboxylase inhibitors, carbidopa and benserazide[6], block almost completely the pupillary response to L-dopa (fig. 2). Carbidopa and benserazide themselves do not have any effect on the pupillary diameter. Fusaric acid, an inhibitor of the enzyme dopamine-beta-hydroxylase[7], reduces the pupillary response to L-dopa by approximately 50% (fig. 2). Concentrations higher than 50 mg/kg produced lethal effects. Phentolamine, the alpha-adrenergic blocking agent, blocked the pupillary response to L-dopa (fig. 2).

Discussion. Our results demonstrate that L-dopa produces dose-dependent mydriasis in mice. However, much higher

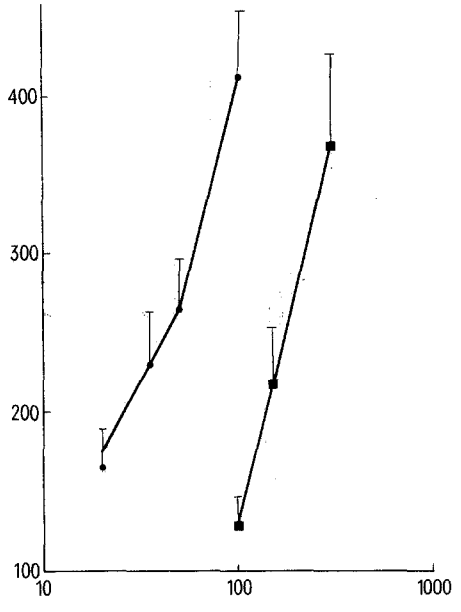

Figure 1. Dose-response curves of dopamine (●) and of L-dopa (■). Abscissa, dose in mg/kg; ordinate, pupil diameter expressed as percentage of pretreatment value. Each point represents the mean (±SEM) of 10 mice.

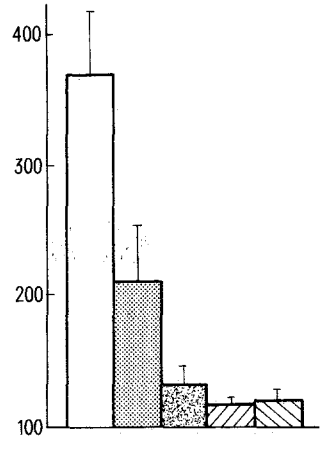

Figure 2. Mydriatic response to 300 mg/kg L-dopa, alone or following various pretreatments. □, 300 mg/kg L-dopa; ▨, pretreatment with 50 mg/kg fusaric acid; ▩, pretreatment with 300 mg/kg benserazide; ▨, pretreatment with 300 mg/kg carbidopa; ▧, pretreatment with 50 mg/kg phentolamine. Bars indicate mean pupil diameters (±SEM) as compared to the values in the naive state. Each bar represents 8 animals, and all pretreatments reduced significantly the L-dopa effect (Student's t-test, $p < 0.05$).

concentrations of L-dopa than of dopamine are needed to achieve any degree of mydriasis (fig. 1).

L-dopa, unlike dopamine, can pass the blood-brain barrier and so may affect pupillary size through central mechanisms. To examine this possibility we injected L-dopa in mice pretreated with peripheral dopa-decarboxylase inhibitors. The use of these agents is expected to increase any central effect of L-dopa. The mydriatic effect of L-dopa was blocked completely by 300 mg/kg carbidopa or benserazide (fig. 2). Thus, the mydriasis produced by L-dopa in mice involves peripheral rather than central mechanisms. The abolition of L-dopa mydriasis by carbidopa precludes the possibility that L-dopa itself directly stimulates postsynaptic receptors. The mydriasis induced following L-dopa injection must therefore occur indirectly, for example after conversion to either dopamine or noradrenaline. Pretreatment with fusaric acid, an inhibitor of the enzyme dopamine-beta-hydroxylase, antagonized the pupillary dilation caused by L-dopa. Although we could not obtain a complete blockade of the mydriasis by fusaric acid (because higher doses of this drug proved to be toxic), the observed inhibition is significant (fig. 2).

Thus, our results indicate that the pupillary dilation produced in mice following the injection of L-dopa is caused by its peripheral conversion to noradrenaline, which in turn stimulates the alpha-adrenergic receptors in the dilator iridis, thus producing mydriasis. The complete blockade by phentolamine of L-dopa induced mydriasis (fig. 2) supports this conclusion. These results complement our recent report on the effects of dopamine in this system[8], which are consistent with the present findings.

Treatment with L-dopa in humans produces several autonomic side-effects[4]. The exact mechanism responsible for these is not clear but it is possible that some (mainly those which are prevented through the concurrent administration of decarboxylase inhibitors) may be underlain by mechanisms similar to those described here. Other effects, such as action on sympathetic ganglia[9], or on presynaptic dopamine receptors in noradrenergic terminals[10,11], may also contribute to autonomic dysfunction following treatment with L-dopa.

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Studies on the in vitro binding of D-penicillamine to cholestyramine

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Summary. Adsorption of D-penicillamine to cholestyramine depends on the amount of the resin, the pH and the presence of other compounds such as bile salts. In the usual drug to resin ratio (150 mg D-penicillamine and 4–8 g cholestyramine per single dose) the percentage of D-penicillamine adsorbed to cholestyramine was about 10% of the applied dose; Bile salts (10 mmoles/l) inhibited this small adsorption by 87%.

The anionic exchange resin cholestyramine[1,2] is used in the treatment of a variety of conditions including the pruritus of cholestatic syndromes[3,4]. It is believed to relieve itching by interruption of the enterohepatic circulation of bile acids[2,5], reducing their concentration in the serum[6]. In addition to bile acids, cholestyramine has been shown to bind a large number of other substances. Interactions of the resin with drugs may have clinical implications, since they may decrease serum levels of these compounds and hence reduce their therapeutic effect. Binding to cholestyramine has been demonstrated for digitalis[7,8] anticoagulants[9,10], antiinflammatory and analgesic drugs[11], antibiotics[12] and a number of other ionic[12] and non-ionic compounds[13]. The anionic drug D-penicillamine is used in patients with primary biliary cirrhosis[14,15] who often need cholestyramine therapy for itching. The potential interaction of cholestyramine with D-penicillamine must, therefore, be regarded as a problem of clinical interest. Although it has been recommended that simultaneous administration of these drugs should be avoided[16], exact information about the binding of D-penicillamine to cholestyramine is missing. Therefore, the binding of D-penicillamine to the resin was studied and the influence of bile salts on this interaction investigated.

Materials and methods. Cholestyramine in anhydrous form and of pharmaceutical grade (Lappe AG, Bergisch-Gladbach, FRG) was dried in a desiccator before use. D-penicillamine (β,β' dimethylcysteine) was generously supplied by Knoll AG (Ludwigshafen, FRG). The bile salts were of analytical grade and obtained from Sigma Chemicals (St. Louis, Mo. USA). The other chemicals were of analytical grade and purchased from commercial sources.

Experimental procedere: Anion-exchange studies were performed according to the method of Gallo et al.[12], slightly modified, by incubation of 5 µmoles D-penicillamine with various amounts (25, 50, 100 mg) of cholestyramine suspended in 5 ml 0.15 M saline (pH 7.7), corresponding to a drug to resin ratio of 1:33 to 1:133. pH dependence was studied from pH 1 to pH 10 with 400 mg cholestyramine and 5 µmoles D-penicillamine. To determine maximal