Potentiation of the central actions of 5-hydroxytryptophan in rabbits by DL-α-hydrazino-α-methyldopa

DL-α-Hydrazino-α-methyldopa (HMD) (Porter, Watson & others, 1962) and RO 4–4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine] (Burkard, Gey & Pletscher, 1964) are effective in potentiating both the central effects of dopa in animals (Bartholini, Blum & Pletscher, 1969; Lotti, 1969) and some of the anti-Parkinson actions in man (Cotzias, Papavasiliou & Gellene, 1969; Tissot, Bartholini & Pletscher, 1969). The mechanism of this potentiation is based on the ability of HMD to inhibit peripheral L-aromatic amino-acid decarboxylase, but presumably not of that in the central nervous system, thus permitting more of the dopa to reach the brain where decarboxylation to dopamine occurs. These findings suggested to us the possibility of using HMD in our experiments on the central actions of DL-5-hydroxytryptophan (5-HTP) in the rabbit, because this amino-acid is also decarboxylated by L-aromatic amino-acid decarboxylase to give 5-hydroxytryptamine (5-HT).

The behavioral and hyperthermic actions of 5-HTP in the rabbit were used as the end points in the experiments now described. 5-HTP, 35–50 mg/kg, intravenously, produced dose-dependent excitation and hyperthermia. We found previously that 75–100 mg/kg of 5-HTP caused extreme excitation, hyperthermia, and death of the animal (Horita & Gogerty, 1958). With the smaller doses of 5-HTP the hyperthermia reached a peak approximately 1 h after administration and gradually returned to normal over the succeeding 2–3 h. HMD alone (25 mg/kg, i.v.) had little effect on rectal temperature. The administration of 35 mg/kg of 5-HTP to 18 HMD-pretreated rabbits produced a mean increase of about 2·5° in rectal temperature in 13 animals, while five of the animals showed either no increase or a slight decrease in rectal temperature under these conditions. The temperature responses of these latter animals are plotted as a separate curve in Fig. 1. In those rabbits responding with hyperthermia, the peak increase occurred between 2 and $2\frac{1}{2}$ h after 5-HTP injection, while in controls given only 5-HTP, responses averaging some 0·6°, occurred within the first hour after injection. The duration of action of the HMD + 5-HTP-treated animals also was consistently longer than in control animals. Five of the

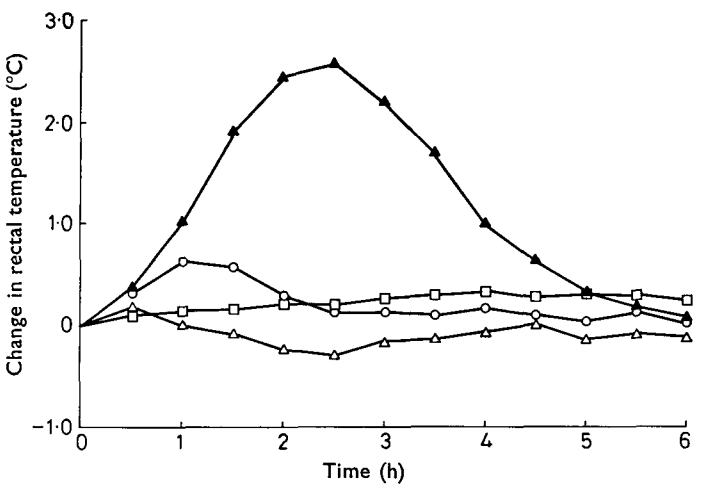

FIG. 1. Effect of 5-HTP on rectal temperature of the rabbit. 5-HTP (35 mg/kg), was administered at 0 h. HMD was given 30 min before 5-HTP administration. ○—control 5-HTP (n = 6); □—control HMD (n = 6); ▲—HMD + 5-HTP, responders (n = 13); △—HMD + 5-HTP, non-responders (n = 5).

Table 1. *Brain levels of* 5-HT *in control,* HMD-, 5-HTP-, *and* HMD + 5-HTP-*treated rabbits*

Treatment	n	5-HT content (μg/g) ± s.d.
a. Control	6	0·50 ± 0·02
b. HMD	6	0·50 ± 0·06
c. 5-HTP	8	0·87 ± 0·15
d. HMD + 5-HTP ..	8	1·47 ± 0·14

HMD was given i.v. in a dose of 25 mg/kg. 5-HTP dosage was 35 mg/kg, i.v., and in group d was administered 30 min after the HMD. Animals were killed 2 h after the 5-HTP injections.

13 responders exhibited temperatures above 43–44° and succumbed during the first 3 h after 5-HTP administration. A dose of 50 mg/kg of 5-HTP administered to HMD-pretreated rabbits produced marked excitation and hyperthermia, the latter rising to above 42°, and in most instances the animals succumbed at the peak of the temperature response.

Brain 5-HT levels were determined in control, HMD-treated, and HMD + 5-HTP-treated animals. Two h after 5-HTP was administered the animals were killed with an intravenous injection of air. The brains were rapidly removed, homogenized in 0·1N HCl, and 5-HT levels were assayed (Bogdanski, Pletscher & others, 1956). Care was taken to employ a double borate buffer wash of the butanol extract to completely remove any residual 5-HTP that might have been extracted. The results of these analyses are in Table 1. In control animals which had 35 mg/kg of 5-HTP 2 h before death, the brain 5-HT concentrations increased 74%, while in HMD-pretreated animals the same dose of 5-HTP produced a 194% increase. These data coincide with potentiation of the excitatory and hyperthermic actions of 5-HTP by HMD. HMD by itself did not affect brain 5-HT concentrations.

Thus HMD is effective in potentiating the central actions of 5-HTP and permits its use in smaller amounts to produce pharmacological responses. Also, the 5-HTP-induced hyperthermia in rabbits appears to be a centrally mediated phenomenon. This suggests the desirability of using HMD rather than a monoamine oxidase inhibitor to potentiate the central actions of 5-HTP, especially since the latter compounds may add further variables to the central nervous system pharmacology of 5-HTP and 5-HT (Green & Sawyer, 1964). Finally, these results point to a possible use for drugs such as HMD as an adjunct to 5-HTP therapy in Down's syndrome (Bazelon, Paine & others, 1967), much as HMD appears to be useful with dopa in alleviating symptoms of Parkinson's Disease.

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Identification of cannabis

The higher incidence of abuse of cannabis in recent years has necessitated identification of larger numbers of cannabis samples. This in turn has caused workers in the field (e.g. Turk, Dharir & Forney, 1969; de Faubert Maunder, 1969a, b, c) to search for more rapid tests with a view to reducing the time of analysis to the minimum that allows certainty of identification. We should like to describe a procedure for analysis that is advantageous in combining two independent techniques for the detection of three cannabis components and which offers positive identification in a reasonably short time (20 min). It consists of extraction of the suspected cannabis or cannabis resin sample with a stock solution of dibenzylphthalate in light petroleum, the extract then being analysed without further purification by gas chromatography and also by paper chromatography. Both chromatographic systems offer good resolution of the three cannabis components.

An extract is prepared by shaking the cannabis or cannabis resin vigorously for 1 min with sufficient stock solution of dibenzylphthalate (10 mg/ml) in light petroleum (40–60°) to produce a mixture containing approximately 20% w/v cannabis or 10% w/v of cannabis resin. The supernatant solution is used, without further purification, for chromatography.

In our experiments a Pye 104 Gas Chromatograph equipped with a flame ionization detector and a Kelvin Electronics servoscribe recorder has been used. The column is glass, 5 ft × 4 mm internal diameter, packed with 80–100 mesh acid-washed, siliconized Diatomite C which is coated with 1% cyclohexanedimethanol succinate (CDMS). A hydrogen pressure of 18 lb/inch^2, air 7 lb/inch^2, and a nitrogen flow rate of 50 ml/min is used throughout. The operating temperature is 220°. 1 μl of the extract is injected onto the column at an appropriate attenuation and the retention times of cannabidiol (CBD), Δ^1-3,4-*trans*-tetrahydrocannabinol (THC), and cannabinol (CBN) are calculated relative to dibenzylphthalate, (DBT) the internal standard. The total analysis time is approximately 15 min. Retention times of the cannabinols relative to dibenzylphthalate are: cannabidiol 0·26; THC 0·39; cannabinol 0·64.

For paper chromatography, Whatman SG81 paper (7 × 25 cm) is immersed in a 15% w/v solution of silver nitrate in distilled water, the excess solution is allowed to drain off, and the paper is then air dried. After applying spots of the extract of suspected cannabis or cannabis resin, and of Δ^1-3,4-*trans*-tetrahydrocannabinol, the paper is developed in chloroform using the ascending technique. Location of the cannabinols is by spraying successively with a 1% solution of Fast Blue Salt B in water and then 2N sodium hydroxide. Development time is 10 min for a 5 cm run. Rf values are: cannabidiol 0·3; THC 0·6; cannabinol 0·8.

A number of gas chromatographic systems for the analysis of cannabis samples have previously been reported, the most recent of which (Lerner, 1969) has described the use of OV.17 as the stationary phase and (±)-methadone hydrochloride as an