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The distribution of Δ^1-tetrahydrocannabinol* and 7-hydroxy-Δ^1-tetrahydrocannabinol in the mouse brain after intraventricular injection

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Δ^1-Tetrahydrocannabinol (Δ^1-THC) and 7-hydroxy-Δ^1-THC were injected into the cerebral ventricles of mice by an improved technique, and the potencies of the drugs were measured by the mouse catalepsy test. Both drugs were found to have the same activity when administered by this route as after intravenous injection. Autoradiographic experiments with tritium-labelled compounds showed that at the time of the peak behavioural effect almost all the injected dose of ^{3}H-Δ^1-THC (1·6 mg kg^{-1}) or ^{3}H-7-hydroxy-Δ^1-THC (0·6 mg kg^{-1}) remained in the intraventricular space and had not penetrated the brain tissue. Δ^1-THC was found to remain in the ventricles after the behavioural effect had disappeared; 3% of the injected dose was still present 2 days after injection of ^{3}H-Δ^1-THC (1·6 mg kg^{-1}).

(—)-Δ^1-Tetrahydrocannabinol ((—)-Δ^1-THC), the major psycho-active constituent of cannabis (Gaoni & Mechoulam, 1964; Grunfeld & Edery, 1969), is rapidly metabolized to 7-hydroxy-Δ^1-THC in several species (Christensen, Freudenthal & others, 1971; Nilsson, Agurell & others, 1970; Ho, Estevez & others, 1972). The metabolism occurs extensively in the liver and lungs (Christensen & others, 1971; Nakazawa & Costa, 1971) but not in the brain (Christensen & others, 1971). As 7-hydroxy-Δ^1-THC is itself pharmacologically active (Wall, Brine & others, 1970) it has been suggested by Ben-Zvi, Mechoulam & Burstein (1970) and Mechoulam (1970) that it is solely responsible for the effects produced after administration of Δ^1-THC, although this has been shown not to be so in mice (Gill, Jones & Lawrence, 1973). In an attempt to resolve the question of whether the cns activity of cannabis was due to Δ^1-THC or its metabolite, Christensen & others (1971) measured the relative potency of Δ^1-THC and 7-hydroxy-Δ^1-THC after intraventricular injection, correctly anticipating that, by administering these compounds directly into the brain, metabolic transformation would be greatly diminished, and that it would be possible to obtain a more accurate estimate of the relative potency than after intravenous injection. They found that 7-hydroxy-Δ^1-THC was 18 times as potent as Δ^1-THC, and concluded that the biological activity of Δ^1-THC was due to its metabolite. However, it has been shown (Gill & Jones, 1972) that at peak response only 0·6% of an intravenously administered dose of Δ^1-THC reached the brain. The results obtained by Christensen & others (1971) indicate that in order to produce a comparable effect it was necessary to instil into the cerebral ventricles a quantity of Δ^1-THC 160$\times$ greater than that shown to be distributed throughout the brain following intravenous injection (Gill & Jones, 1972).

* Δ^9-tetrahydrocannabinol according to IUPAC rules.

This suggested that effects other than intrinsic activity at the active site were important, and the experiments described here repeat some of the work of Christensen & others (1971) and supplement it by an autoradiographic determination of the distribution of tritium-labelled Δ^1-THC and 7-hydroxy-Δ^1-THC following intraventricular injection.

MATERIALS AND METHODS

The syntheses of ^{3}H-Δ^1-THC and of both labelled and unlabelled 7-hydroxy-Δ^1-THC have been described elsewhere (Gill & Jones, 1972a; Gill & others, 1973). Unlabelled Δ^1-THC was isolated from the natural material (Gill, 1971).

Injection of drugs into the ventricular system was achieved by holding the mouse in a specially designed jig, which maintained the head in a fixed position. Penetration of the ventricles was by insertion, to a predetermined depth, of a 27 gauge hypodermic needle which was mounted on the jig. The drugs were injected through this needle from an Agla micrometer syringe. By this method small volumes of solution (of the order of 1 μl) could be introduced into the ventricular system, in the area of the 3rd ventricle, with high reproducibility. The alignment of the needle was checked by injection of Indian ink followed by removal and sectioning of the brain. When correctly set the ink was distributed throughout the ventricles within 5 min.

Male albino mice (23–27 g, Tuck strain no. 1 obtained from A. J. Tuck) were injected with Δ^1-THC (0·2, 0·4, 0·8 or 1·6 mg kg^{-1}) or 7-hydroxy-Δ^1-THC (0·1, 0·2 or 0·6 mg kg^{-1}) dispersed in physiological saline (1 μl per 25 g weight) with Tween 80 (7 mg kg^{-1}). The immobility indices of the mice were measured by the mouse catalepsy test (Pertwee, 1972) 15, 45 min, 1·5 and 3 h after injection of the highest dose of each drug, and 15 min after injection of all other doses.

In the autoradiographic experiments the mice were injected with ^{3}H-Δ^1-THC (1·6 mg kg^{-1}) or ^{3}H-7-hydroxy-Δ^1-THC (0·6 mg kg^{-1}) and their immobility indices were measured immediately before death and removal of the intact brain 15 min, 4 h and 2 days after injection of ^{3}H-Δ^1-THC, and 15 min after injection of ^{3}H-7-hydroxy-Δ^1-THC. Autoradiographs of sections of the brains were taken on Ilford G5 emulsion, together with positive and negative chemography controls (Ullberg, 1954); the plates were exposed for 4 weeks at $-5°$.

Whole brain levels of radioactivity were measured 2 days after injection of ^{3}H-Δ^1-THC (1·6 mg kg^{-1}) by scintillation counting as described previously (Gill & Jones, 1972).

RESULTS

The injection procedure itself stunned the mice for about 2 min but they then recovered rapidly. The immobility index of mice injected with 1 μl of saline or Tween 80 (7 mg kg^{-1}) in saline had returned to baseline (21 $\pm$ 4 and 23 $\pm$ 3 respectively; mean of 6 mice $\pm$ s.e.) within 15 min after the injection, and subsequently showed no significant variation over a period of 4 h. The immobility index of mice injected with Δ^1-THC (1·6 mg kg^{-1}) was 68 $\pm$ 4 (mean of 6 mice $\pm$ s.e.), approximately the same as that after intravenous administration of the same dose (Gill & others, 1973). Although the effect of the injection prevented the determination of the exact time of the peak effect, it occurred between 5 and 15 min after the injection, and the

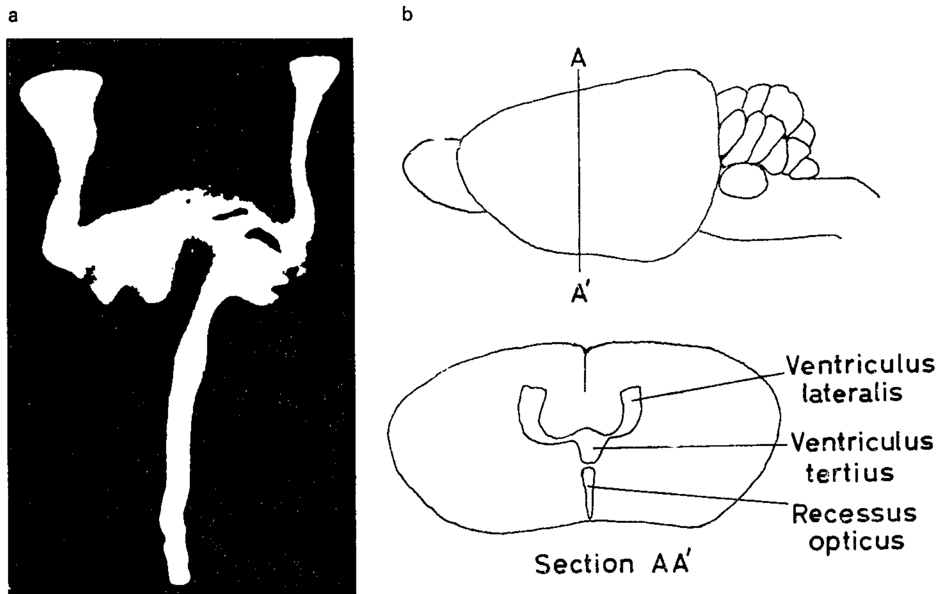

FIG. 1. a. Autoradiograph of a coronal section of mouse brain 20 min after intraventricular injection of ^{3}H-Δ^{1}-THC (1·6 mg kg^{-1}). Only the area enclosing the ventricles is shown as no other features, even the outline of the brains, were visible.

b. Drawing showing the position of the section within the brain (between sections 221 and 231 in Sidman, Angevine & Pierce, 1971) and an impression of the whole section.

behavioural changes persisted for 3–4 h, corresponding closely to the pattern of activity observed after intravenous injection of Δ^{1}-THC (2 mg kg^{-1}; Gill & Jones, 1972). 7-Hydroxy-Δ^{1}-THC was found to be about twice as potent as Δ^{1}-THC when injected intraventricularly and it had a similar duration of action.

The autoradiographs obtained 20 min after injection of ^{3}H-Δ^{1}-THC (1·6 mg kg^{-1}) and ^{3}H-7-hydroxy-Δ^{1}-THC (0·6 mg kg^{-1}) showed that when the behavioural effect was maximal, almost all the radioactivity present in the brain was contained within the intraventricular space (Fig. 1). In addition, autoradiographs taken 4 h and 2 days after the injection of ^{3}H-Δ^{1}-THC showed that a considerable amount of radioactive material was still present in the ventricles, even though the animals had fully recovered, and no behavioural effect could be observed.

The chemical nature of the radioactive material present in the brain was determined by extraction of the homogenized brain with ethyl acetate, and identification by thin-layer chromatography and scintillation counting (Gill & Jones, 1972). Two days after the injection of Δ^{1}-THC, when about 3% of the injected dose remained in the ventricles, the extractable material (1·5% of the injected dose) was found to consist almost entirely of Δ^{1}-THC (94%); less than 3% of the activity could be attributed to 7-hydroxy-Δ^{1}-THC. A further 1·5% of the injected dose was present as non-extractable material. This experiment gives only a minimum value for the level of radioactivity, since some cerebrospinal fluid was always lost during removal of the brain. However, the amount of Δ^{1}-THC found in the ventricles after 2 days was sufficient (3 nmol g^{-1} brain) to produce a marked effect if evenly distributed throughout the brain (Gill & others, 1973).

DISCUSSION

The injection of cannabinoids directly into the brain appears at first sight to be a simple and reliable method to obtain the potency ratios for these compounds, since the absence of metabolism in the brain itself causes the levels of metabolites to be much lower than after intravenous administration (Christensen & others, 1971). The contribution of active metabolites to the activity of the injected drug is thus greatly reduced.

This method was used by Christensen & others to estimate that 7-hydroxy-Δ^1-THC was about 18 times as potent as Δ^1-THC in producing behavioural changes in mice. The doses used in those experiments, however, were approximately the same as those required to produce the same behavioural change by intravenous injection. Gill & Jones (1972) showed that after intravenous injection of ^{3}H-Δ^1-THC in mice only 0·6% of the injected dose reached the brain, which suggests that after intraventricular injection only a small fraction of the dose penetrated to the site of action.

In the present work Δ^1-THC was found to be approximately equipotent by intravenous and intraventricular administration. This result agrees well with that obtained by Christensen & others (1971) even though those workers used different criteria in assessing the behavioural effect (Irwin, 1968), and injected a volume of 30 μl, which is about the same as the total volume of the cerebrospinal fluid in the mouse brain. However, in the present work, it was found that 7-hydroxy-Δ^1-THC was about twice as potent as Δ^1-THC after intraventricular injection, whereas Christensen & others (1971) obtained a value of 18 times. This discrepancy could arise if the peak effect due to the metabolite occurred less than 15 min after injection, since in these experiments, reliable immobility index readings were unobtainable earlier due to the effect of the injection itself. Christensen & others quoted results 10 min after injection. The agreement between the two groups on the activity of Δ^1-THC suggests, however, that differences in the injection technique could not completely account for this discrepancy. Taken at its face value, the low potency ratio found for 7-hydroxy-Δ^1-THC, together with the relatively small amounts found in the brain following intravenous injection of Δ^1-THC, would suggest that, contrary to the assertion of Christensen & others (1971), the metabolite is not solely responsible for the action of Δ^1-THC in mice. However, the autoradiographic studies on the distribution of Δ^1-THC or its metabolite after intraventricular injection show that the simple potency ratios measured must be interpreted with great caution.

Previous work (McIsaac, Fritchie & others, 1971; Freudenthal, Martin & Wall, 1972) has shown that after intravenous administration, Δ^1-THC is quickly distributed throughout the brain. The autoradiographs taken at varying periods after intraventricular injection showed that the Δ^1-THC was almost completely contained within the ventricles, and that only a minute amount diffused from the intraventricular space. This observation accounts for the fact that, in order to produce comparable effects, the same dose is required given intraventricularly, as is required when given intravenously. The apparent relative potencies observed are as much a function of the ability of either Δ^1-THC or its metabolite to diffuse from the intraventricular space, as it is of their true relative potencies.

Most substances in true solution, when injected into the cerebral ventricles, are dispersed rapidly, and lipid-soluble molecules, in particular, partition rapidly into the surrounding brain tissue; colloidal solutions and suspensions, on the other hand, remain localized for much longer (Davson, 1956). Δ^1-THC is a very lipid-soluble

compound and is very insoluble in water. In order to get a sufficiently high concentration in an aqueous medium for animal administration it is necessary to use a non-ionic dispersing agent, and in this work, in common with several other groups of workers (e.g. Christensen & others, 1971; Ho & others, 1972), Tween 80 was used. The Δ^1-THC injected into the ventricles was therefore not in true solution, but was present almost entirely in Tween 80/THC micelles. When such a dispersion is injected intravenously there is presumably a rapid equilibration of the Δ^1-THC from the micelles to the plasma lipoproteins and possibly the chylomicra, and from these phases it apparently penetrates the blood-brain barrier easily and quickly. The cerebrospinal fluid is a plasma ultrafiltrate, practically devoid of macromolecular constituents, and in this medium the Tween stabilized micelles appear to yield up their Δ^1-THC very slowly to the walls of the ventricles. Recent work (Paton, unpublished) has shown that the partition ratio of Δ^1-THC between red cell ghosts and saline, which normally favours the membrane by a ratio of 1600:1, is greatly reduced by the addition of Tween 80; at about 2% v/v Tween 80 the ratio is 1:1. Intraventricular injection, therefore, cannot be used to give meaningful potency ratios for cannabinoids that do not readily dissolve in water.

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REFERENCES

BEN-ZVI, Z., MECHOULAM, R. & BURSTEIN, S. (1970). *J. Am. chem. Soc.*, **92**, 3468–3469.

CHRISTENSEN, H. D., FREUDENTHAL, R. I., GIDLEY, J. T., ROSENFELD, R., BOEGLI, G., TESTINO, L., BRINE, D. R., PITT, C. G. & WALL, M. E. (1971). *Science*, **172**, 165–167.

DAVSON, H. (1956). *Physiology of Occular and Cerebrospinal Fluids.* 14 *et seq.* & 174. London: Churchill.

FREUDENTHAL, R. I., MARTIN, J. & WALL, M. E. (1972). *Br. J. Pharmac.*, **44**, 244–249.

GAONI, Y & MECHOULAM, R. (1964). *J. Am. Chem Soc.*, **86**, 1646–1647.

GILL, E. W. (1971). *J. chem. Soc. (C)*, 579–582.

GILL, E. W. & JONES, G. (1972). *Biochem. Pharmac.*, **21**, 2237–2248.

GILL, E. W. & JONES, G. (1972a). *J. Labell. Compounds*, **8**, 237–248.

GILL, E. W., JONES, G. & LAWRENCE, D. K. (1973). *Biochem. Pharmac.*, **22**, 175–184.

GRUNFELD, Y & EDERY, H (1969). *Psychopharmacologia*, **14**, 200–210.

HO, B. T., ESTEVEZ, V., ENGLERT, L. F. & MCISAAC, W. M. (1972). *J. Pharm. Pharmac.*, **24**, 414–416.

IRWIN, S. (1968). *Psychopharmacologia*, **13**, 222–257.

MCISAAC, W. M., FRITCHIE, G. E., IDANPAAN-HEIKKILA, J. E., HO, B. T. & ENGLERT, L. F. (1971). *Nature*, **230**, 593–594.

MECHOULAM, R. (1970). *Science*, **168**, 1159–1166.

NAKAZAWA, K. & COSTA, E. (1971). *Nature*, **234**, 48–49.

NILSSON, I. M., AGURELL, S., NILSSON, J. L. G., OHLSSON, A., SANDBERG, F. & WAHLQVIST, M. (1970). *Science*, **168**, 1228–1229.

PERTWEE, R. J. (1972). *Br. J. Pharmac.*, **46**, 753–763.

SIDMAN, R. L., ANGEVINE, J. B. Jr. & PIERCE, E. T. (1971). *Atlas of the Mouse Brain and Spinal Cord*, Harvard University Press.

ULLBERG, S. (1954). *Acta Radiol.*, Stockholm, Supplement 118.

WALL, M. E., BRINE, D. R., BRINE, G. A., PITT, C. G., FREUDENTHAL, R. I. & CHRISTENSEN, H. D. (1970). *J. Am chem. Soc.*, **92**, 3466–3468.