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The metabolism and distribution of pemoline in rat brain*

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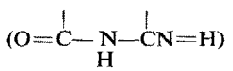
RECENT experiments with pemoline (2-imino-4-keto-5-phenyloxazolidine) dissolved in undiluted dimethylsulfoxide (DMSO) and administered intraperitoneally (i.p.) to rats have shown both elevated brain levels of this drug[1] and facilitation of maze learning[2] relative to the effects found when the drug was administered in an aqueous vehicle.[3] The known ability of DMSO to enhance permeability of biological membranes to a variety of solutes[4] may have accounted for these findings.

The detection of measurable quantities of ^{14}C-labeled pemoline (iminophenyloxazolidinone-2-^{14}C)† in the brain raised the question of a possible differential distribution of the drug in various brain structures which could be related to its behavioral effects in rats.[2]

Assay for pemoline-^{14}C in brain. Fourteen male albino rats weighing 400–450 g were injected i.p. with PIO-^{14}C (0·1 μc/μmole) dissolved in DMSO (6·75 mg/ml) at a dose level of 4·5 mg/kg in a volume of 0·67 ml/kg. One hr after injection, the rats were sacrificed by thoracic exsanguination to drain the brain of blood as completely as possible. The brain was exposed after decapitation and the different regions were collected by suction into test tubes with side arms connected to a fine-tip aspirator. The tubes were kept in crushed ice and the following brain areas were collected in cold isotonic saline as quickly as possible: anterior cortex including frontal poles, posterior cortex including occipital areas, caudate nucleus, amygdala and septum, hippocampus, corpus callosum and fimbria, thalamus, brainstem and cerebellum. The segments were transferred to preweighed centrifuge tubes and centrifuged at 1000 *g* for 5 min in the cold. The supernatant saline was removed as completely as possible and the precipitate weighed. Segments were homogenized in 0·5 or 1 ml H_2O and samples of the homogenate were assayed for radioactivity in a Packard liquid scintillation counter as described by Mahin and Lofberg.[5] To test for possible metabolic degradation products of PIO-^{14}C, whole brains were homogenized 2 hr after drug administration in an equal volume of cold 1 M $HClO_4$. After centrifugation, the supernatant solution was neutralized with 6 M KOH to pH 8 and kept at 0° to precipitate $KClO_4$. The solution obtained after removal of $KClO_4$ by centrifugation was reduced *in vacuo* and chromatographed on Whatman No. 1 paper with *n*-propanol:H_2O:conc. NH_4OH (88:10:2) after addition of carrier PIO to the spots. Radioactivity was located by dividing the chromatogram into 1-cm^2 segments above the origin and each section was mixed with 15 ml of scintillation solution (toluene:methyl cellosolve:2,5diphenyloxazole; 10:6:0·06, v/v/w) for counting. All the radioactivity in whole brain extracts thus scanned after chromatography was localized to a single spot having the same mobility as an authentic sample of PIO-^{14}C (R_f = 0·71). This solvent was employed to obtain a separation of PIO from creatinine (R_f = 0·30) and to locate compounds having a chromophore



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† Pemoline-^{14}C was a gift from Drs. H. G. Schoepke and J. C. Netwal of the Pharmacology Department of Abbott Laboratories and was diluted to a specific activity of 0·1 μc/μmole with recrystallized iminophenyloxazolidinone from the Aldrich Chemical Company. Abbreviation used: Pemoline, PIO.

similar to that of creatinine. Pemoline and creatinine on chromatograms could then be visualized by spraying them with an alkaline picrate solution which reacted with both compounds to give orange spots (Jaffe reaction). Urinary excretion of PIO-^{14}C was assayed in a similar manner, after collecting urine into pads of Whatman filter paper followed by elution with 95% ethanol, and chromatographed for counting as described above. Radioactivity was excreted in urine at a linear rate of approximately 1 per cent of the administered dose per hr for 3 hr, in which an unidentified metabolic product was detected which had an R_f value of 0·5 and represented approximately 14 per cent of the total radioactivity excreted in 2 hr, the remainder being unchanged PIO-^{14}C.

Distribution of PIO-^{14}C in brain. An analysis of the radioactivity in the different brain regions of the rats is shown in Table 1 where the results are expressed as the mean and S.E.M. for 14 rats. The

TABLE 1. DISTRIBUTION OF PEMOLINE^{14}C IN THE BRAIN OF RATS AFTER INTRAPERITONEAL ADMINISTRATION IN DMSO*

	Brain regions						
No.†	Anterior cortex	Posterior cortex	Caudate nucleus	Hippo-campus	Thalamus	Brainstem	Cerebellum
14	1·21 ± 0·07	1·18 ± 0·09	1·16 ± 0·09	1·10 ± 0·07	1·23 ± 0·09	1·40 ± 0·11	2·61 ± 0·08

* Values are expressed as micrograms per gram wet weight (mean ± S.E.M.).
† Number of animals used for each assay.

average amount of PIO in the combined brain areas of aroused rats was 1·48 ± 0·20 μg/g wet wt. (mean ± S.D.) and for gentled rats, 1·34 ± 0·21 μg/g. These values are slightly lower than those found for whole brain, since areas such as lateral and ventral cortex and residual white matter were not included in the assay.[1] It is unlikely that the amounts represent any significant contamination by residual blood in the brain vascular space (calculated to be approximately 3·5 per cent of the brain volume of the rat),[6] since blood levels of PIO-^{14}C at 60 min were approximately of the same order of magnitude (3·9 μg/ml blood vs. 4·5 μg for whole brain).[1]

No statistically significant differences in levels of radioactivity are seen between any of the cerebral areas analyzed. The cerebellum appears to be the most active region for accumulation of the drug, since approximately twice as much radiopemoline is found in this region than in any of the observed cerebral areas. The relevance of these findings to the mode of action of pemoline in influencing cognitive[2] or other behavioral parameters remains to be elucidated.

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