smooth muscle provides a unique method for determining the action of suspected vasodilating agents. The method has particular merit for evaluating agents with limited aqueous solubility.

*Résumé.* Des sections de muscle lisse d'aorte de lapin ou d'artère fémorale de chien répondent à une stimulation de champ (AC) par une contraction due en partie à la libération de catécholamines, contraction pouvant être relâchée sous l'effet de divers médicaments. D'autre part, l'AC manifeste une action au niveau de la membrane excitable et au-delà de cette membrane. Il en a peut-être aussi sur le couplage excitation-contraction. L'AC offre une méthode unique pour l'étude des agents susceptibles d'avoir une action vaso-dilatatrice.

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## An Assay Procedure for Mescaline and its Determination in Rat Brain, Liver and Plasma

Trimethoxyphenylethylamine or mescaline is a known hallucinogenic compound in man[1] and produces abnormal behavior in animals[2]. Recently the behavioral effects of mescaline were compared with those obtained after the administration of similar methoxylated phenylethylamines[3,4]. Since the concentrations of most of these compounds in the CNS are unknown these structure-activity relationships had to be based on quantities injected. In order to measure the concentrations of mescaline in the CNS after injection of the compound, a rapid assay procedure was developed and the brain, liver, and plasma levels of this compound were determined as a function of time and dose in male Sprague-Dawley rats weighing approximately 200 g.

The assay procedure is based on the extraction of mescaline from biological tissues and fluids and on its reaction with dansyl chloride. The tissue (approximately 1–2 g) was homogenized in 3 volumes of 1 *N* HCl. To the homogenate or plasma (1.0 ml of plasma and 3 ml of 1 *N* HCl), 1.5 ml of 5 *N* NaOH and 30 ml of toluene were added. After shaking and centrifugation for 10 min, 25 ml of toluene were removed and shaken with 1.5 ml of 0.5 *M* boric acid for 10 min. After centrifugation for 10 min, 1 ml of the boric acid was combined with 1 ml of 0.1 *M* borax solution and 0.02 ml of dansyl chloride (10 mg/ml of acetone) and heated in a boiling water bath for 15 min. After cooling the samples were shaken with 1.5 ml of chloroform for 10 min, and the organic phase was read in an Aminco-Bowman Spectrofluorophotometer at 490 nm (activation 338 nm). Tissue samples from untreated animals with and without the addition of a known amount of mescaline served as internal standards or blanks, respectively.

The sensitivity of the procedure is approximately 0.5 µg/g or ml of sample and the recovery is between 65 and 80%. Fluorescence of extracts obtained from untreated animals was only slightly higher than that of 'water' blanks. Verification of the specificity of the method was obtained by thin layer chromatography[5] and by the BRODIE distribution[6].

After i.p. injection of mescaline (Table I), the compound appeared rapidly in plasma and liver and most of it disappeared within 2 h. Mescaline entered the brain slowly, showed peak levels at 30 min, and could still be detected after 210 min. The ratio of the concentrations in brain and plasma at peak levels was approximately 0.6. An increase in the dose injected (Table II) produced higher plasma and tissue levels with the exception of brain in which saturation was reached at a dose of 40 mg/kg. In separate experiments it was shown that brain levels of mescaline did not exceed 2.7 mg/g 60 min after injection of 80 mg/kg. These results might indicate that mescaline does not cross the blood-brain-barrier easily and is stored in the CNS at specific sites with a limited capacity.

Our data agree with those of DENBER and TELLER[7] who found cortical peak levels of approximately 1 µg/g 40 min after the injection of 10–18 mg/kg into female

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Table I. Concentrations of mescaline in rat brain liver, and plasma as a function of time

| | Min after injection | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 15 | 30 | 60 | 120 | 210 |
| | µg/g or ml | | | | | |
| Brain | n.d. | 2.1 ± 0.4 | 3.2 ± 0.7 | 1.5 ± 0.9 | 1.25 ± 0.5 | 1.4 ± 0.8 |
| Liver | 35.5 ± 6.8 | 31.3 ± 5.9 | 22.1 ± 2.1 | 6.8 ± 2.2 | 3.0 ± 0.3 | – |
| Plasma | 3.5 ± 0.3 | 3.1 ± 0.8 | 4.9 ± 2.0 | 1.9 ± 1.0 | n.d. | – |

Each value is the mean ± the standard deviation from at least 3 animals. Rats received 40 mg/kg of mescaline × hemisulfate i.p. n.d., not detectable.

rats. In contrast, the injection of 25 mg/kg of mescaline into cats produced peak levels in the brain of 4 μg/g after 30–60 min and the brain-plasma ratio was approximately 4[8]. Injection of the same quantity of mescaline into dogs produced cortical levels of 13 μg/g after 4 h and the brain-plasma ratio was approximately 2[9]. These data could indicate a species difference in the accumulation of mescaline in the CNS.

The physiological disposition of 4-methoxyphenylethylamine (M-1) and 3,4-dimethoxyphenylethylamine (M-2) in the rat is known[10,11]. A comparison of these data with those obtained with 3,4,5-trimethoxyphenylethylamine (M-3) or mescaline in this paper shows the following differences: First, after the injection of comparable amounts, M-1, M-2, and M-3 can be detected in the brain for approximately 30, 45, and at least 210 min. Second, the injection of increasing doses of M-1 and M-2 produces an increase in brain levels, whereas M-3 shows saturation in the CNS. Third, peak levels after the injection of 40 mg/kg i.p. of M-1, M-2, and M-3 are approximately 17, 8, and 3 μg/g, respectively. Ratios of the concentrations in the brain and plasma are approximately 4, 2, and 0.6, respectively. The number of methoxy groups seems to decrease the penetration of these compounds into the CNS. Fourth, the presence of M-1 and M-2 in the CNS correlates fairly well with the time of abnormal behavior whereas no such correlation is apparent for M-3; rats appear normal after approximately 2 h[4] although brain levels of M-3 are still as high as at times of abnormal behavior. Finally, the smallest doses of M-1, M-2, and M-3 which will show abnormal behavior in rats are approximately 40, 25, and 12.5 mg/kg, respectively. At these doses brain levels of M-1, M-2, and M-3 are approximately 17, 3, and 0.5 μg/g, respectively. A comparison of the injected doses of M-1 and M-3 shows that M-3 is approximately 3 times more potent than M-1. However, a comparison of the brain levels of both compounds at these doses reveals that M-3 produces abnormal behavior at 1/34 the concentration at which M-1 is effective[12].

Table II. Concentrations of mescaline in rat brain, liver and plasma as a function of dose injected

| | Dose (mg/kg) 20 | 40 | 80 |
|---|---|---|---|
| | μg/g or ml | | |
| Brain | 0.9 ± 0.4 | 3.2 ± 0.7 | 2.7 ± 0.6 |
| Liver | 6.1 ± 4.2 | 22.1 ± 2.1 | 52.5 ± 13.8 |
| Plasma | 2.6 ± 1.0 | 4.9 ± 2.0 | 10.2 ± 1.9 |

Each value is the mean ± the standard deviation from at least 3 animals. Rats were killed 30 min after i.p. administration of mescaline × hemisulfate.

*Zusammenfassung.* Eine Schnellmethode zur Bestimmung von Meskalin in Geweben und biologischen Flüssigkeiten wird beschrieben. Nach der Injektion (40 mg/kg, i.p.) sinken die Konzentrationen von Meskalin in Leber und Plasma während der folgenden 2 h rasch ab, während die Verbindung im Gehirn (ca. 1,5 μg/g) bis zu 3,5 h unverändert verbleibt. Ein Vergleich zwischen Meskalin und zwei verwandten Verbindungen wurde angestellt.

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## N-(t-Aminoalkynyl)-Substituted Pyrrolidones as Oxotremorine Antagonists

The high degree of muscarinic potency of oxotremorine, 1-(2-oxopyrrolidino)-4-pyrrolidino-2-butyne, peripherally[1] as well as centrally[2], has stimulated the interest in structural modifications of this agent. A number of compounds closely related to oxotremorine have been synthesized by BEBBINGTON et al.[3] and NEYMEYER et al.[4], but it was found that all structural changes resulted in compounds which were either less active than oxotremorine or inactive.

In 1966 we observed that replacement of the 2-oxopyrrolidino moiety of the oxotremorine molecule by a succinimide group led to compounds with antagonistic properties[5,6], and it was found later that slight modifications in the 2-butyne chain could lead to compounds which were about one hundred times more active as antagonists than the parent compound[7]. We found it of interest to investigate how similar modifications of the 2-butyne chain of oxotremorine would influence the pharmacological properties, and we now wish to report on the synthesis and pharmacological properties of a series of compounds closely related to oxotremorine in which the 2-butyne chain has been branched with 1 or 2 methyl groups or lengthened with 1 methylene group between the acetylenic bond and the lactam

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