The Distribution of Morphine Following Intracerebral Microinjection

Recently the hypothermic effect of morphine was studied using the technique of intracerebral microinjection[1]. The principle factor limiting precise localization of the site of action in these studies was the question of the spread of the drug away from the injection site. This problem has been investigated using C^{14}-labelled morphine.

Method. Morphine N-methyl-C^{14} with a specific activity of 3.5 μc/mg was converted to the sulphate and made up to a final concentration of 50 μg/μl in 0.9% NaCl. Injection of 1 μl was made into the anterior hypothalamus of the rat using a sterotaxic instrument and a microliter syringe with a needle of 0.07 mm O.D. mounted in the electrode carrier.

The animals were killed with pentobarbital, the brains removed and blocks containing the injection site sectioned at 100 μ intervals on a freezing microtome. Alternate sections were placed in counting vials and the tissue digested by agitation with 0.5 ml of 0.5 *N* KOH for 60 min at 25 °C. 18 ml of 'Liquifluor'/methyl alcohol/toluene scintillation mixture were added to the vials and the radioactivity was counted on a Packard Tri-Carb counter. A vial containing 50 μg of morphine sulphate and a section of brain tissue was treated in the same way and used as an internal standard to check on the performance of the counter. The counts were normalized in respect of this standard for each experiment.

Results. Injections were made into 3 rats immediately after death and the brains sectioned within a few minutes. The mean activity in these animals is seen at the top of Figure 1. After correction for the sections discarded, the total activity in these animals approximated to 100% of the counts from the standard tube indicating that the losses in the technique are small and that the morphine had remained at the site of injection. It can be seen that the morphine has spread for approximately 1 mm from the center and that over 90% of the activity is less than this distance from the center. In a second group of animals injected post-mortem the brains were left in situ for 60 min or longer before removal and sectioning. All of the radioactivity remained at the injection site and the profile of the distribution was indistinguishable from that in the brains sectioned immediately after injection, indicating that diffusion away from the injection site does not occur.

24 animals were sacrificed 10, 20, 40, or 60 min after intracerebral injection; the distribution of the radioactivity in the sections and the number of animals in each group is seen in Figure 1. The spread of activity is virtually the same at each time interval and not significantly different from that in animals injected after death, again indicating that diffusion through the tissues does not occur to any measurable extent. A steady decline in the total counts occurs as the drug is removed in the blood stream. In Figure 2 the percentage activity has been plotted (using the counts from the post-mortem group as 100%) at the various time intervals and, assuming first order kinetics, the best line fitted to the points. This indicates a half-life following intracerebral injection of about 20 min.

12 further rats killed 2, 6, or 24 h after injection showed little or no radioactivity in the sections.

Discussion. A volume of 1 μl would be contained in a sphere approximately 0.6 mm in radius – close to the distribution seen in the present study. Thus it would appear that the spread of the morphine following intracerebral injection depends on the volume of the injection.

The drug is rapidly removed by the blood stream following injection into the brain. It is likely that all of the receptors within the injection site are initially occupied and that the excess molecules of morphine act as a buffer preventing rapid clearance of the receptors. Such a

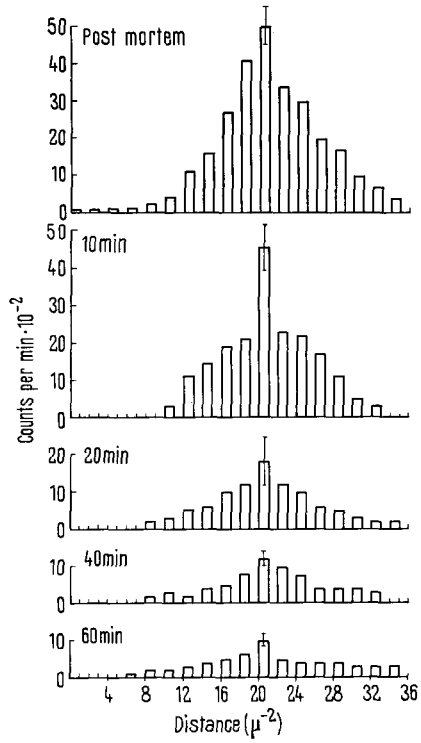


Fig. 1. Radioactive counts in alternate 100 μ sections following injection of 50 μg of C^{14}-morphine into the anterior hypothalamus. The injections were made post-mortem (3 rats) and 10 min (8 rats), 20 min (6 rats), 40 min (4 rats) and 60 min (6 rats) before sacrificing the animals. Vertical bars represent standard errors of the means.

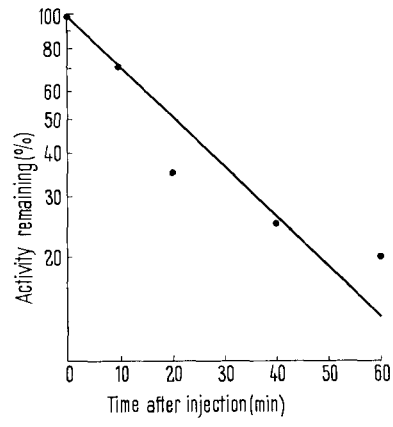


Fig. 2. Total radioactivity in brain sections at various time intervals after intracerebral injection of 50 μg of C^{14}-morphine. Counts from animals injected after death taken as 100%. Points represent means of number of animals noted in Figure 1.

[1] V. J. Lotti, P. Lomax, and R. George, J. Pharmacol. *150*, 135 (1965).

hypothesis could account for the much higher tissue concentration when intracerebral is compared with systemic administration. In the rat it was found that 50 μg of morphine sulphate injected into the thermoregulatory centers gave a mean hypothermic response equivalent to 25 mg/kg intravenously[2]. The concentration of morphine in the brain of the rat following intravenous injection of 25 mg/kg was 3.4 μg/g wet weight[3]. The blood concentration following systemic administration is 3–4 times greater than the brain levels and would tend to maintain the concentration at the receptors. The initial rate of fall in temperature is the same with intracerebral doses ranging from 10–100 μg but the degree of hypothermia developing is dose dependent, indicating that the duration of action is the limiting factor[4].

It is possible that the lack of free diffusion is due to the physico-chemical properties of the brain tissue with its closely packed cellular elements and paucity of interstitial fluid space. No extracellular fluid space could be demonstrated in the rat cortex using electron microscopy[5] and it was suggested that transport in brain tissue is across astrocytic cells. Post-mortem swelling of the brain cells, however, may account in part for these appearances[6].

The technique of intracerebral injection of drugs would seem to provide a degree of localization comparable to the use of electrolytic lesions or stimulation of the brain[7].

Résumé. La distribution de la morphine radioactive après une injection intracérébrale de cette drogue a été étudiée. Les résultats indiquent que la drogue ne se diffuse pas du site de l'injection à travers le tissu cérébral. La morphine est enlevée par le sang et a une période de demi-vie dans le cerveau d'environ 20 min.

P. LOMAX

Department of Pharmacology and Brain Research Institute, University of California, Los Angeles (California, USA), December 7, 1965.

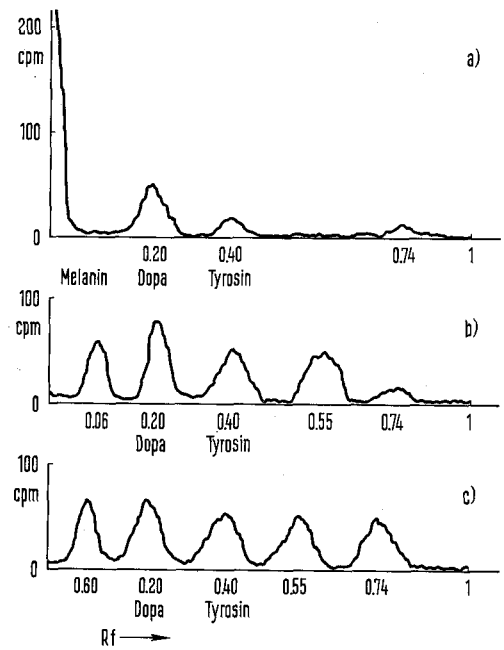
[2] V. J. LOTTI, P. LOMAX, and R. GEORGE, Int. J. Neuropharmacol. (1965), in press.
[3] J. C. SZERB and D. H. MCCURDY, J. Pharmacol. *118*, 446 (1956).
[4] V. J. LOTTI, P. LOMAX, and R. GEORGE, Int. J. Neuropharmacol. (1965), in press.
[5] R. L. SCHULTZ, E. A. MAYNARD, and D. C. PEASE, Am. J. Anat. *100*, 369 (1957).
[6] A. VAN HARREVELD, J. cell. comp. Physiol. *57*, 101 (1961).
[7] This research was supported by NSF Grant GB-1484.

Inhibition of Phenoloxidase Catalysed Transformation of Tyrosine to Melanin by Hydroxyphenylcarboxylic Acids

The significance of the presence of the enzyme phenoloxidase as well as melanin in certain regions of the mammalian central nervous system (CNS) is still unclear[1,2]. What has been definitely established is that disorders of melanogenesis are found in certain neurological disorders such as Parkinsons disease and oligophrenia phenylpyruvica[3,4]. In oligophrenia phenylpyruvica, an accumulation of phenylcarboxylic acids has been noted, but this is still unsuccessfully correlated with the pathogenesis of the disease. In the present paper, an inhibitory action of *p*-hydroxyphenylpyruvic and 3,4-dihydroxyphenylpyruvic acid on tyrosine oxidation to melanin by phenoloxidase, leading to accumulation of intermediate metabolites with potential toxic action on the nerve cells, will be described.

Materials and methods. Due to difficulties in obtaining phenoloxidase from brain in sufficient quantities, we used a preparation derived from the insect *Calliphora erythrocephala*[5].

10 Calliphora larvae were homogenized in 10 ml of 0.25 *M* sucrose containing 1% dehydroascorbic acid adjusted to pH 7. The homogenate was centrifuged 10 min at 1200 *g* and the supernatant thus obtained 10 min at 7000 *g*. The sediment was suspended in 0.25 *M* sucrose and centrifuged once more for 10 min at 7000 *g*. The sediment



Oxidation of tyrosine by phenoloxidase. (a) without inhibitor; (b) in the presence of $5.5 \cdot 10^{-3}$ *p*-hydroxyphenylpyruvic acid; (c) in the presence of $5.5 \cdot 10^{-3}$ 3,4-dihydroxyphenylpyruvic acid. Incubation time: 10 min at 37 °C. Paper chromatograms of the incubation mixtures in butanol/*n*-HCl.

[1] C. V. WENDE and M. T. SPOERLEIN, Life Sci. *6*, 386 (1963).
[2] G. C. COTZIAS, P. S. PAPAVASILIOU, M. H. VAN WOERT, and A. SAKAMOTO, Fedn. Proc. Socs exp. Biol. *23*, 713 (1964).
[3] J. W. HEATH, Archs Neurol. Psychiat. *58*, 484 (1947).
[4] J. A. N. CORSELLIS, J. Neurol. Neurosurg. Psychiat. *16*, 139 (1953). – J. H. FELLMANN, J. Neurol. Neurosurg. Psychiat. *21*, 58 (1958).
[5] C. E. SEKERIS and D. MERGENHAGEN, Science *145*, 68 (1964).