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Cellular and metabolic tolerance to an opioid narcotic in mouse brain

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Summary

1. Running activity and brain levorphanol concentration were measured in nontolerant and tolerant mice given various doses of ^{3}H-levorphanol.

2. The principal factor responsible for tolerance in the mouse is a loss of sensitivity to the narcotic drug at the cellular level in brain; despite adequate brain concentrations, the pharmacological effects are diminished or absent.

3. There is also metabolic tolerance; a given dose establishes a lower brain concentration in tolerant than in non-tolerant animals.

4. The two kinds of tolerance are distinguished here and the contribution of each is assessed.

Introduction

The studies of Mule & Woods (1962) in the dog and of Mule, Redman & Flesher (1967) in the guinea-pig showed that tolerance to morphine was not due to a reduction of the morphine level in brain. Richter & Goldstein (1970) demonstrated that in tolerant mice, ordinary doses of levorphanol produced neither analgesia nor running activity despite the establishment of sufficiently high drug concentrations in the brain water. Here we examine some quantitative aspects of the relationship between dose, brain levorphanol concentration, and running activity in non-tolerant and tolerant mice.

Methods

Swiss-Webster mice of both sexes were used (Simonsen Laboratories, Gilroy, Calif.) weighing 20–35 g. All were prescreened for adequate running response to a 20 mg/kg i.p. injection of levorphanol tartrate (dose expressed in terms of free base). This and all subsequent procedures for measuring running activity and for producing tolerance were exactly as described by Goldstein & Sheehan (1969).

For experiments with non-tolerant mice, ^{3}H-levorphanol was given in a single injection. For tolerant mice, the last injection of the series was ^{3}H-levorphanol. A continuous record of running activity for each mouse was obtained for 30 min after injection, then the animal was decapitated. The running rate just before decapitation was taken as the criterion of drug effect, to be related to the drug concentration in the brain at that time. At each dose and condition, 7 to 21 animals were used (median=9), except that in the non-tolerant group there were only 3 mice at the 80 mg/kg dose.

Each brain was removed quickly and homogenized in 2 ml of cold 5% trichloroacetic acid (TCA), and 1 ml of TCA was used for rinsing into a centrifuge tube. The TCA supernatant was adjusted to pH 10·8 (shown to be optimum) and

extracted with 5 vols of benzene for 5 min (shown to be sufficient). Aliquots of each phase were taken for scintillation counting. The TCA pellet was resuspended in water, adjusted to pH 10·8, extracted with benzene, and aliquots of both phases were counted. The aqueous phase containing TCA-insoluble material that was not extracted into benzene was solubilized with hydroxide of Hyamine (Packard Instrument Co., Downers Grove, Ill.). The remainder of each benzene extract was evaporated to dryness, taken up in methanol and spotted on thin-layer chromatography plates (Eastman Chromatograms, No. 6061, without fluorescent indicator) that had been presoaked in 0·2 M glycine–0·2 M NaCl, pH 10·4. These were devoleped in chloroform:methanol:glycine buffer, pH 10·4, 80:20:10 v/v. In this system the Rf of levorphanol is $0{\cdot}81 \pm 0{\cdot}05$ (S.E.) and that of norlevorphanol $0{\cdot}47 \pm 0{\cdot}01$. Levorphanol and norlevorphanol standards were included as internal markers in each run and were identified by spraying with Kiefer's reagent (10 ml of 1% potassium ferricyanide and 2–3 drops 1 N ferric chloride). Scintillation counting was carried out in a naphthalene-xylene-dioxane fluid (Carey & Goldstein, 1962) and internal standards (^{3}H-toluene or ^3H_2O) were used to determine counting efficiencies.

Results and Discussion

Figure 1 shows the relationship of running activity to the brain concentration of apparent levorphanol (radioactivity), and Table 1 shows that a very large fraction of the radioactivity, both in non-tolerant and tolerant mice, appeared to be levorphanol.

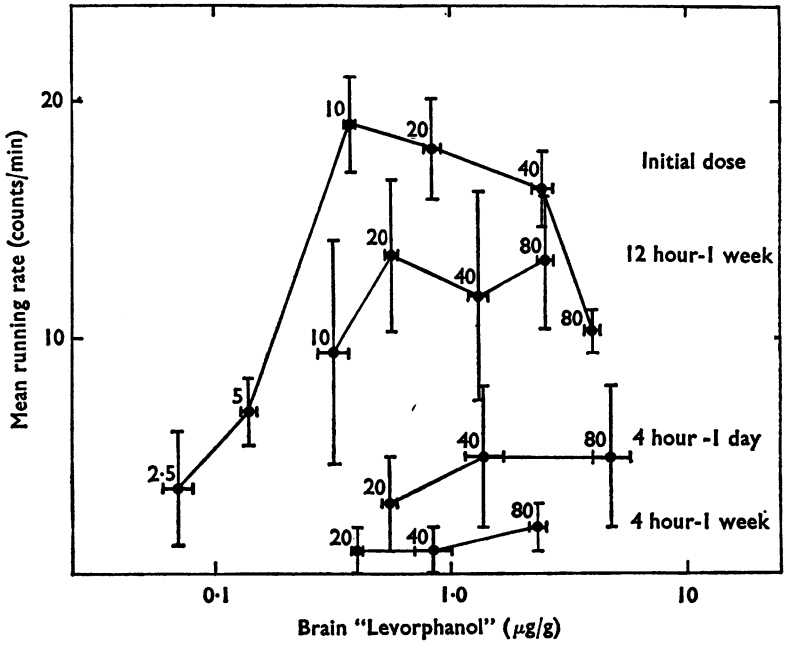

FIG. 1. Relationship between brain radioactivity (apparent levorphanol) and running activity. Data are means ± S.E.M. Running rate was obtained from the cumulative record at the time of decapitation. Uppermost curve represents nontolerant animals, lower three curves are for animals maintained on 20 mg/kg doses of levorphanol at the interval and for the duration shown. The number next to each point is the dose of ^{3}H-levorphanol given 30 min prior to decapitation. For further details see **Methods.**

TABLE 1. *Identification of levorphanol in mouse brain*

	Initial dose (10 mg/kg)	Initial dose (20 mg/kg)	Tolerant (20 mg/kg)
Number of mice	3	6	4
TCA supernatant	0·59±0·04	1·00±0·18	0·63±0·04
Extractable by benzene	70%±2%	82%±4%	75%±2%
Amount in the benzene extract with Rf* of			
Levorphanol	0·27 (>93%)†	0·58 (>92%)†	0·27 (>79%)†
Norlevorphanol	<0·01	0·04	0·04
At origin	<0·01	<0·01	0·03
TCA insoluble residue	—	0·97±0·21	0·59±0·02
Extractable by benzene	—	80%±3%	72%±6%
Amount in the benzene extract with Rf of			
Levorphanol	—	0·47 (>73%)†	0·40 (>95%)†
Norlevorphanol	—	0·15	<0·01
At origin	—	0·02	<0·01

The conditions examined here correspond to the two most effective initial doses of levorphanol (non-tolerant mice) in Fig. 1, and to a similar dose that was virtually without effect in tolerant mice (20 mg/kg, at 4 h intervals). Unless otherwise indicated, data are μg (±S.E.M.)/g of fresh brain, computed from dpm and the specific radioactivity of the ^{3}H-levorphanol. When authentic levorphanol was added to TCA supernatant or to TCA insoluble residue, 94–97% was extractable into benzene under these conditions.

* By thin-layer chromatography, see **Methods**. † Percent of the amount recovered at these three positions on the TLC plate.

In non-tolerant mice, the maximum effect was obtained at a dose of 10 mg/kg, which established a concentration of 0·37±0·03 μg/g. At 20 mg/kg the effect was about the same, but at higher doses it diminished, as depressant actions supervened. The corresponding curves for tolerant mice show that with increasing frequency of injection, the running activity at any given brain concentration decreased. In addition, a given dose of levorphanol produced a lower brain concentration than in non-tolerant animals. Thus, the principal basis of tolerance was a loss of sensitivity to levorphanol at the cellular level in brain, but metabolic tolerance was also present. We shall show elsewhere that the metabolic tolerance is due to increased conjugation and excretion of levorphanol.

About one-quarter of the radioactivity in brain was not extractable by benzene, i.e., was a polar derivative of levorphanol. A measurable but small fraction (<1%) appeared to be covalently bound to the TCA-insoluble tissue residue (not shown in Table 1). These metabolites of levorphanol in brain, some of which changed in relative amount in the tolerant state, are under further investigation.

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