Failure of morphine dependence in rats to influence brain noradrenaline turnover

SIR,—Since Vogt (1954) showed that morphine depleted the level of noradrenaline in the cat brain there have been many studies in which an attempt has been made to show a relation between morphine dependence and catecholamine metabolism. The effects of single doses or chronic administration of morphine on brain noradrenaline levels are complex and depend on the dose of drug and on the species of animal used.

Chronic administration of morphine to rats usually produces a small increase in brain noradrenaline levels (Freedman, Fram & Giarman, 1961; Maynert & Klingman, 1962; Gunne, 1963; Akera & Brody, 1968), but in the dog there is no increase in brain catecholamine levels (Maynert & Klingman, 1962; Gunne, 1963). When rats are abruptly withdrawn from long-term chronic morphine administration there is a withdrawal syndrome which is not associated with a decrease in brain noradrenaline, but in the dog, which has a more excitatory type of withdrawal syndrome, a large fall in noradrenaline level is seen in the brain after morphine withdrawal.

The significance of the raised brain noradrenaline levels in the rat is not known. This effect of chronically administered morphine is not dose dependent as is the severity of the abstinence syndrome and does not occur with levorphanol or methadone (Akera & Brody, 1968). Maynert & Klingman (1962) and Gunne (1963) found that the injection of a monoamine oxidase inhibitor into control and chronically treated rats produced a greater increase of brain noradrenaline in the morphine-tolerant animals and they suggested that this might indicate an increase in the synthesis of brain noradrenaline.

In the present experiments, noradrenaline turnover in morphine-dependent rats and in animals during drug withdrawal has been estimated from the rate of disappearance of radioactive noradrenaline from the brain (Iversen & Glowinski, 1965).

Sprague-Dawley rats initially weighing between 190–210 g were given two daily injections of morphine hydrochloride at 9.00 and 17.00 hr for 4 weeks. The doses were increased at the end of each week starting at 20 mg/kg and increasing to 50, 150 and finally 250 mg/kg intraperitoneally, control rats were given equivalent volumes of 0·9% saline solution. After four weeks, noradrenaline turnover in half the chronically treated and half the control rats was estimated. The morphine injections in the remaining chronically treated rats were replaced by saline injections. The noradrenaline turnover in these animals and in the remaining controls was estimated 60 hr after the withdrawal of morphine.

The rats were lightly anaesthetized with ether and killed at various times after the intracisternal injection of DL[^{3}H]noradrenaline hydrochloride (specific activity 1·82 c/mmole), 5 μc in 50 μl of Merle's solution. The brains were rapidly removed onto a chilled surface, weighed and homogenized in 14 ml of 0·4 N perchloric acid containing 20% EDTA (1%) at 0–4°, and left to extract for 45 min. After centrifugation, 0·2 ml of the supernatant fluid was transferred into a vial containing 4·0 ml of ethoxyethanol and 10 ml of phosphor (0·01% POPOP and 0·4% DPO in toluene) and the total radioactivity estimated by liquid scintillation counting. The remaining supernatant fluid was assayed for [^{3}H]noradrenaline and [^{3}H]normetanephine after ion-exchange chromatographic separation using Amberlite CG-120, Type 2. ^{3}H-Deaminated metabolites were calculated by difference (Iversen, 1963). Endogenous noradrenaline was estimated in some samples by the fluorometric method of Euler & Lishajko (1961).

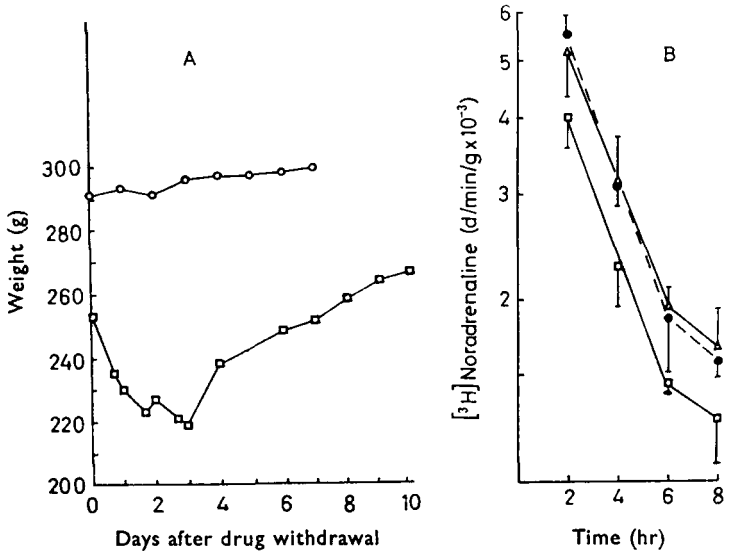

FIG. 1A. The effect of morphine withdrawal upon body weight of rats after chronic administration of an increasing dose of morphine twice a day. At day zero, saline was substituted for morphine (□). Controls (O). Each point is the mean body weight of 18 rats.

B. Disappearance of [³H]noradrenaline from whole brain after intracisternal injection. Controls (●). Morphine dependent (△). Morphine withdrawn (□). Each point is the mean of four or five rats. The vertical lines show the s.e. of the mean.

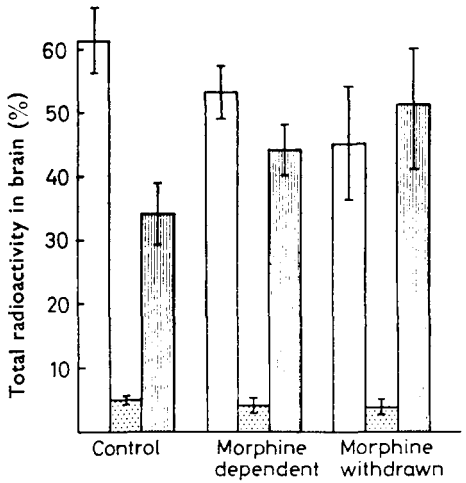

FIG. 2. Patterns of metabolites of [³H]noradrenaline in the whole rat brain 2 hr after intracisternal injection. Open columns, [³H]noradrenaline. Stippled columns, [³H]normetanephrine. Hatched columns, ³H-deaminated metabolites. Each value is expressed as a percentage of the total radioactivity in the brain and is the mean of three to five determinations. Vertical lines are the s.e. of the mean.

During the first week, injections of morphine into rats caused increased activity which lasted for about 1 hr. This effect was not apparent in the last week, by which time the rats had become somewhat vicious and difficult to handle. The withdrawal of morphine produced a pronounced abstinence syndrome in rats which was most obvious after 48 hr. The animals became very irritable, difficult to handle and showed hyperalgesia, piloerection and tremor. Diarrhoea and anorexia occurred with a subsequent mean loss of body weight of 13% after 3 days (Fig. 1A). Similar abstinence syndromes in rats have been described by Hanna (1960), Gunne (1961), and Akera & Brody (1968).

TABLE 1. ENDOGENOUS NORADRENALINE LEVEL OF RAT BRAIN

Group	Concentration μg/g ± s.e.	Number of rats
Control	0·43 ± 0·03	6
Morphine dependent	0·47 ± 0·06	7
Withdrawn	0·47 ± 0·03	5

Noradrenaline turnover in rat brain was not affected by the chronic administration of morphine or by withdrawal of morphine from tolerant rats (Fig. 1B). The half-lives for noradrenaline turnover in control ($t\frac{1}{2}$ = 2·3 hr), morphine dependent ($t\frac{1}{2}$ = 2·9 hr) and "withdrawn" rats ($t\frac{1}{2}$ = 2·6 hr) did not differ significantly and are similar to the results of Glowinski, Kopin & Axelrod (1965) who found the half-life of [^{3}H]noradrenaline in whole rat brain to be about 3 hr. Although the rats were undoubtedly morphine dependent, the endogenous noradrenaline levels of morphine dependent and "withdrawn" rats were not significantly greater than in controls (Table 1).

There was no significant difference between control, morphine dependent or "withdrawn" rats in the pattern of [^{3}H]noradrenaline metabolites (Fig. 2). In all groups about 50% of the radioactivity in the brain after 2 hr was noradrenaline, this proportion increased to about 75% after 8 hr. As in the experiments of Glowinski & others (1965) the major fraction of metabolites was present as ^{3}H-deaminated products.

It appears from the present experiments that noradrenaline turnover in rat brain is not increased by morphine dependence or withdrawal of the drug. The increase in brain noradrenaline level reported by most authors is apparently not essential for the production of drug dependence in rats, for although the animals in the present experiments showed an obvious abstinence syndrome, there was no significant increase in brain catecholamine levels. Morphine dependence in rats produced by dose schedules which increase brain noradrenaline levels, does not increase brain 5-hydroxytryptamine levels (Maynert, Klingman & Kaji, 1962; Gunne, 1963) which presumably means that there is no inhibition of monoamine oxidase. This is supported by the present failure of chronic morphine treatment to significantly alter the pattern of catecholamine metabolism in the rat brain. As it seems unlikely that the increased levels of brain noradrenaline in morphine dependent rats are produced by increased synthesis or by inhibition of monoamine oxidase, it may be that chronic morphine administration affects the intraneural storage mechanisms of catecholamines. This suggestion is supported by the experiments of Freedman & others (1961) who found that morphine temporarily and partially reverses the noradrenaline releasing property of reserpine.

Acknowledgements. My thanks are due to Mr C. N. Rayner and Mr R. D. Stretch for their technical assistance.

Department of Pharmacology, M. J. NEAL
Cambridge University, Cambridge, England.
October 2, 1968

References

Akera, T. & Brody, T. M. (1968). *Biochem. Pharmac.*, **17**, 675–688.
Euler, U. S. von & Lishajko, F. (1961). *Acta. physiol. scand.*, **51**, 348–355.
Freedman, D. X., Fram, D. H. & Giarman, N. J. (1961). *Fedn Proc.Fedn Am. Socs exp. Biol.*, **20**, 321.
Glowinski, J., Kopin, I. J. & Axelrod, J. (1965), *J. Neurochem.*, **12**, 25–30.
Gunne, L. M. (1961). *Psychopharmacologia*, **2**, 214–220.
Gunne, L. M. (1963). *Acta physiol. scand.*, **58**, Suppl. 204, 5–91.
Hanna, C. A. (1960). *Archs int. Pharmacodyn. Ther.*, **124**, 326–329.
Iversen, L. L. (1963). *Br. J. Pharmac. Chemother.*, **21**, 523–537.
Iversen, L. L. & Glowinski, J. (1965). *J. Neurochem.*, **13**, 671–682.
Maynert, E. W. & Klingman, G. I. (1962). *J. Pharmac. exp. Ther.*, **135**, 285–295.
Maynert, E. W., Klingman, G. I. & Kaji, K. (1962). *Ibid.*, **135**, 296–299.
Vogt, M. (1954). *J. Physiol., Lond.*, **123**, 451–481.

An apparatus for the study of intestinal transfer of drugs

SIR,—An *in vitro* method for the study of intestinal transfer suitable for use with radioactive labelled compounds is described. An outline of the apparatus is illustrated in Fig. 1. A segment of small intestine is tied to a cannula at each end and suspended vertically in an organ bath with its oral end upwards so that any peristaltic contractions propel fluid in the direction of the circulation. Fluid to be perfused through the intestinal lumen (mucosal fluid) is added to the reservoir and its circulation is started by raising the reservoir initially to allow filling of the connecting tubings, and is maintained by a continuous stream of 5% carbon dioxide in oxygen. This gas mixture also serves to keep the pH constant at 7·4 and supply the oxygen requirements of the mucosal layer of the intestine. The serosal aspect of the intestine is bathed in fluid (serosal fluid) of composition similar to the mucosal fluid except that it contains the substance being studied. The serosal fluid is aerated with the same gas mixture introduced through a thin polythene tubing. The apparatus as illustrated is immersed in a thermostatic tank at 38°.

To mount the intestine, the lower cannula, consisting of a silicone tubing held by a rubber bung, is lightly coated with silicone fluid to facilitate its sliding within the lumen of the bung. The anal end of the intestine is then tied to the silicone tubing and the oral end of the intestine "threaded" into the organ bath. The silicone tubing is pushed into the organ bath until the oral end of the intestine protrudes from the bath and can be tied to the glass cannula. The silicone tubing is then retracted sufficiently to allow the preparation to be totally immersed in fluid. The reservoir can be raised or lowered to provide the desired intraluminal pressure as measured by the difference in level of the mucosal and serosal fluids (see Fig. 1). An intraluminal pressure of 2 cm or more will initiate peristaltic contractions (with guinea-pig small intestine). If peristaltic contractions are not required, the intraluminal pressure should be just high enough to allow a constant circulation of mucosal fluid to be maintained. The rate of the circulation can be adjusted by regulating the flow of the gas mixture and circulation time can be varied from 20 to 60 sec. A thin smear of silicone antifoam prevents frothing at the serosal and mucosal fluid surfaces in the organ bath and reservoir respectively.

This simple method permits the economical use of labelled compounds and also provides for efficient oxygenation which is vital in *in vitro* studies. We