Effect of 6-hydroxydopamine on catecholamine concentrations and behaviour in the morphine-tolerant rat

Although many studies on the role of the central adrenergic nervous system in morphine analgesia have been made, no definitive relation has been established. Chronic morphine administration may affect the intraneural catecholamine storage mechanisms, since morphine prevents reserpine-induced depletion of noradrenaline in acute (Freedman, Fram & Giarman, 1961) and chronic studies (Gunne, 1963). Furthermore, chronic administration of morphine interferes with the depletion of brain dopamine as well as brain noradrenaline after reserpine (Gunne, Jonsson & Fuxe, 1970), as determined by fluorescent microanalysis of the nerve terminals. The administration of 6-hydroxydopamine (6-OHDA) into the brain or the lateral ventricles destroys central adrenergic neurons, as determined histochemically (Ungerstedt, 1968; 1971), electronmicroscopically (Bloom, Algeri & others, 1969) and neurochemically (Uretsky & Iversen, 1970; Breese & Traylor, 1970, 1971). Although the mechanisms of 6-OHDA action have not been completely clarified, the compound, being chemically similar to noradrenaline may be taken up and accumulated into the adrenergic neurons by the axonal membrane pump. This would be consistent with the findings that 6-OHDA-induced depletion of monoamines is blocked by protriptyline (Evetts & Iversen, 1970), desipramine or low temperature (Jonsson & Sachs, 1970).

The purpose of the present investigation was to study the effect of acute or chronic administration of morphine on the brain catecholamine and behaviour changes induced by the injection of 6-OHDA into the lateral ventricle of the rat brain.

To obtain rats tolerant to morphine, male Wistar rats (Royal Hart strain), 250 g were injected intraperitoneally twice daily (8 a.m. and 5 p.m.) with morphine sulphate (10 mg/kg; calculated as free base), as an initial dose. The dose of morphine was then increased every 3 days to 20, 40, 60 and finally to 80 mg/kg twice daily. The animals were maintained at the last dose level for a week, and 6-OHDA was then administered. The animals were killed at various times thereafter. Morphine analgesic ED50 was determined by the tail-flick method (Nakamura, Kuntzman & Conney, 1972: unpublished observations). To destroy central adrenergic neurons, 50 or 300 μg of 6-OHDA HBr dissolved in 10 μl of ice-cold saline containing 10 μg ascorbic acid was administered into the right lateral ventricle, without anaesthesia, through an implanted cannula (Nakamura, Gerold & Thoenen, 1971). Brain noradrenaline or dopamine was adsorbed on activated alumina (Anton & Sayre, 1962) and determined fluorimetrically (Shellenberger & Gordon, 1971). Increased irritability induced by 6-OHDA was quantitatively measured by behaviour scores in which the maximum score for the highest degree of irritability was 12 (Nakamura & Thoenen, 1972). The number of animals for each determination was usually 6–8.

The analgesic ED50 (with 95% confidence limits) for intraperitoneal administered morphine in naive rats or animals chronically treated with morphine was 2·7 (1·7–3·8) mg/kg (i.p.) and 19·5 (12·9–26·1) mg/kg (i.p.), respectively (activity measured at time of maximum effect). The brain concentrations of noradrenaline and dopamine in morphine-tolerant rats were slightly higher 24 h after the last dose of morphine being 385 $\pm$ 11 ng/g and 477 $\pm$ 25 ng/g respectively as compared to 359 $\pm$ 9 ng/g and 406 $\pm$ 23 ng/g in control rats. The intraventricular administration of 6-OHDA (50 and 300 μg) caused a marked depletion of brain noradrenaline in control rats and in rats treated either chronically or with a single dose of morphine (Fig. 1). In contrast, as has previously been shown by Bell, Iversen & Uretsky (1970), the administration of 6-OHDA to control rats increased the level of brain dopamine (Fig. 1). The difference in the effect of 6-OHDA on noradrenaline and dopamine seen 24 h after

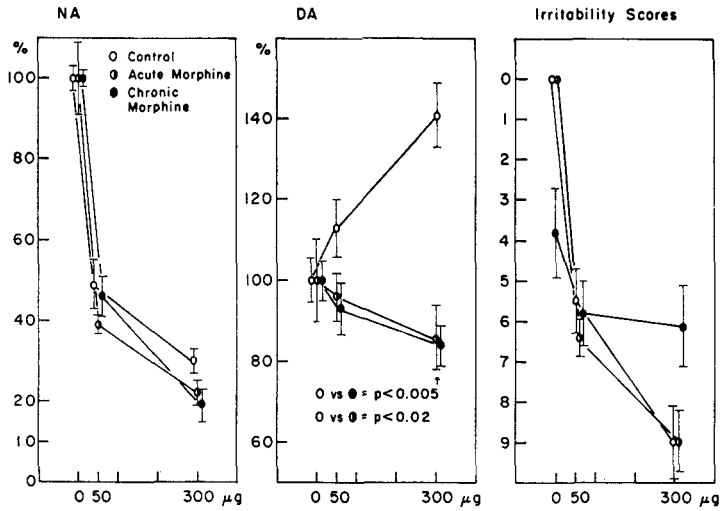

FIG. 1. Effect of acute or chronic administration of morphine on the brain catecholamine changes and increased irritability induced by an intraventricular administration of 6-OHDA. For acute morphine administration, 20 mg/kg of morphine was administered i.p. 1·5 h before the intraventricular administration of 6-OHDA (50, 300 μg). For chronic morphine administration, the animals were injected i.p. with morphine twice a day beginning with 20 mg/kg daily and increasing the dose every three days until the dose reached 160 mg/kg daily. 6-OHDA was injected into the lateral ventricle 1·5 h after the morning dose of morphine. The animals were killed 24 h after the administration of 6-OHDA. Morphine was administered to the chronic morphine rats for the scheduled evening dose. Each value represents the percent of initial concentration with a standard error of mean, calculated from 6 animals. The brain noradrenaline concentration in the control, acutely morphinized, or chronically morphinized animals was 355 ± 9, 327 ± 39 or 385 ± 11 ng/g, respectively, and the brain dopamine content was 406 ± 23, 465 ± 52 or 477 ± 25 ng/g, respectively.

6-OHDA administration may be explained on the basis of localization of these amines in the brain. Both the noradrenergic nerve terminals in the hypothalamus and the noradrenergic cells bodies in the locus coeruleus are in periventricular regions. Although the dopaminergic cell bodies in the substantia nigra have no contact with the ventricles, the dopaminergic nerve endings do. Transient dopamine accumulation in the brain after 6-OHDA, as also observed by fluorescent microscopy (Ungerstedt, 1971), may be due to interruption of an axoplasma flow transport of monoamine granules from the cell bodies to the terminals (Dahlström & Fuxe, 1964). The increase in brain dopamine seen 24 h after the administration of 6-OHDA to control rats was not observed when 6-OHDA was administered to rats previously treated with morphine and, in fact, a decrease in brain dopamine was observed in these rats (Fig. 1). Four days or one week after treatment with 6-OHDA, both brain noradrenaline and dopamine concentrations decreased markedly. While chronic treatment with morphine caused no significant effect on the depletion of brain noradrenaline after 6-OHDA, chronic treatment with morphine did inhibit the depletion of brain dopamine (Fig. 2). The intraventricular administration of 6-OHDA causes increased irritability in rats, as described previously (Nakamura & Thoenen, 1972). The acute administration of morphine (20 mg/kg, i.p.) 1·5 h before 6-OHDA did not prevent the increased irritability (Figs 1 and 2). However, in rats chronically treated with morphine, there was a significant ($P < 0{\cdot}05$) reduction in the increased irritability (Figs 1 and 2) induced by 6-OHDA, in spite of the irritability which resulted from the chronic administration of morphine. These results suggest that a relation may exist between the inhibition by chronic morphine treatment of the 6-OHDA-induced depletion of brain dopamine and increased irritability.

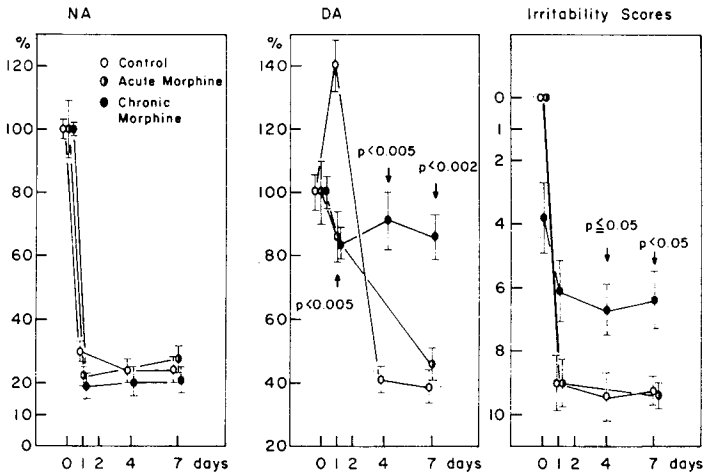

FIG. 2. Effects of chronic administration of morphine on the time-course of the brain catecholamine changes and increased irritability induced by the intraventricular administration of 6-hydroxydopamine. The methods are given in the legend of Fig. 1 and in the text, except that the animals were killed 1, 4 or 7 days after the administration of 6-OHDA (300 μg). Morphine was administered to the chronic morphine treated rats for an additional 4 or 7 days after 6-OHDA.

The mechanism through which chronic morphine treatment inhibits the activity of 6-OHDA on the dopaminergic neurons is unknown. Studies with protriptyline revealed that it prevents 6-OHDA-induced lesions in noradrenaline-containing neurons but not in dopamine-containing neurons, and protriptyline selectivity interferes with the depleting effect of 6-OHDA on noradrenergic neurons, with less effect on dopaminergic neurons (Evetts & Iversen, 1970; Breese & Traylor, 1971). Tolerance to the cataleptic effects of morphine occurred more quickly than did tolerance to morphine analgesia. Furthermore, it is known that a single administration of morphine increased the turnover of dopamine in brain neurons, while chronic treatment of morphine caused no effect on dopamine turnover (Gunne, Jonsson & Fuxe, 1969). The administration of morpine-induced motor excitation in morphine-tolerant rats. The present results suggest that chronic treatment with morphine may induce changes in the uptake process of the nigrostriatal system, eventually inhibiting the uptake of 6-OHDA, because an intraneural uptake of 6-OHDA seems to be a prerequisite for the degeneration effects (Thoenen & Tranzer, 1968; Malmfors & Sachs, 1968).

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Department of Biochemistry and Drug Metabolism,
Hoffmann-La Roche Inc.,
Nutley, New Jersey 07110, U.S.A.

K. NAKAMURA*
R. KUNTZMAN
A. MAGGIO
A. H. CONNEY

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* On leave from Nippon Roche Research Center, Kamakura, Japan.

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Antagonism by INPEA of isoprenaline-induced tachycardia in man

The compound (±)-1-(*p*-nitrophenyl)-2-(isopropylamino) ethanol (±-INPEA) has been shown to have β-adrenoceptor blocking properties in animals (Murmann & Gamba, 1966) and to differ from many existing β-adrenoceptor blocking agents in its associated pharmacological actions. Due to the lack of data on the β-adrenoceptor blocking actions of (±)-INPEA in man, a study was undertaken of its effects on isoprenaline-induced tachycardia in healthy volunteers.

Five fully informed healthy volunteers whilst lying down, had a cannula placed in the antecubital vein for infusion of isoprenaline and injection of (±)-INPEA. The electrocardiogram was monitored and recorded continuously from the CR5 lead; heart rate was counted from the recording. The subjects received initially an injection of physiological saline and the heart rate response to this injection was observed for 5 min. At least 5 min after the injection of saline, isoprenaline was infused by means of a mechanically-driven syringe at a rate of 3 μg/min for 4 min and the heart rate response was recorded during and after the infusion until basal conditions had been re-established. Thirty min after the infusion of isoprenaline a single dose of (±)-INPEA was administered and approximately 5 min later the infusion of isoprenaline was repeated.

Isoprenaline sulphate B.P. was dissolved in isotonic sodium chloride injection containing 0·003% ascorbic acid to yield a final concentration of 1·5 μg per ml. A fresh solution was made for each subject. (±)-INPEA was used as an aqueous solution containing 25 mg per ml.

The subjects received 10, 25 and 45 mg of (±)-INPEA. The only side effect was peripheral paraesthesiae which was noted by one subject after intravenous administration of 45 mg.

Infusion of isoprenaline 3 μg/min for 4 min increased the heart rate in all five subjects, (increased rate control: 29·0 ± 2·4, after (±)-INPEA: 10 mg, 22·6 ± 4·3; 25 mg, 13·6 ± 1·4*; 45 mg, 12·2 ± 2·0* means ±s.e. of 5 subjects).

The increase had stabilized between the third and fourth minute of the infusion and this increase at the end of the fourth minute was taken as the response to isoprenaline. The mean response to the infusion of isoprenaline for the five subjects was an increase of 29 beats/min (±2·4 s.e.).

* Significantly different from control $P<0·001$