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THE EFFECTS OF MICROIONTOPHORETICALLY APPLIED MORPHINE AND TRANSMITTER SUBSTANCES IN RATS DURING CHRONIC TREATMENT AND AFTER WITHDRAWAL FROM MORPHINE

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The effects of microiontophoretically applied acetylcholine, noradrenaline, 5-hydroxytryptamine and morphine were studied on single brain stem neurones of rats during chronic morphine pretreatment and 24 h after its withdrawal. No significant changes were observed in the initial spontaneous neuronal firing rate or in the qualitative or quantitative effects of acetylcholine, noradrenaline or 5-hydroxytryptamine. However, in chronically treated animals there was a significant decrease in the number of neurones excited by morphine or showing tachyphylaxis to morphine on repeated microiontophoretic applications.

We suggest that some of the cellular central nervous system changes which occur during chronic morphine treatment are reflected by the decrease in sensitivity of neurones to morphine excitation.

It has been suggested that cellular changes in the central nervous system, particularly changes in the disposition and turnover of neurotransmitters (Clouet, 1971) or in receptor sensitivity (Collier, 1965; 1968) may account for the tolerance and physical dependence which results from chronic administration of morphine. We have used the technique of microiontophoresis to study possible changes in the sensitivity of single brain stem neurones to morphine and to certain putative neurotransmitters (acetylcholine, noradrenaline and 5-hydroxytryptamine), in rats following the chronic administration of morphine and also after its withdrawal.

Methods Adult albino rats (350-600 g) were individually housed under the same environmental conditions and received the same diet except for the contents of their drinking fluid. Control animals were given 45% sucrose solution for 21 days. Morphine-treated animals received increasing amounts of the drug (0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml over successive 7 day periods) dissolved in 45% sucrose solution. A further group of animals was treated with morphine in the same way but the drug was replaced by sucrose solution 24 h before the experiment. All animals were allowed to drink these solutions *ad libitum* and their behaviour, fluid intake and body weight were monitored.

Following pretreatment the animals were anaesthetized with urethane (1.2-2.4 g/kg) and the cerebellum removed to expose the floor of the 4th ventricle. Spontaneous extracellular activity of single neurones in the pons-medulla was recorded through the central barrel (4 M NaCl) of a five-barrelled glass micropipette. Another barrel contained 1 M NaCl to monitor current effects, and the remaining barrels contained solutions of the following drugs, at the indicated concentrations and pH: acetylcholine chloride, 0.3 M, pH 4.0-5.0 (Sigma); (−)-noradrenaline bitartrate, 0.12 M, pH 5.0-6.0 (Sigma); 5-hydroxytryptamine (serotonin) bimaleinate, 0.2 M, pH 4.5-5.5 (Koch-Light); morphine hydrochloride, 0.03 M, pH 4.0-5.0 (Macfarlan Smith Ltd).

The effects of the three neurotransmitters, applied in the same order, with a current of 20 nA for 20 s, were tested on single cells in the control animals and the results compared with the morphine-pretreated and morphine-withdrawn animals. A retaining current of 15 nA was used in each case and each compound was applied only once to each cell to avoid tachyphylaxis. Comparisons were made between animals in each pretreatment group and their controls, of (a) the absolute number of spikes occurring during a drug response; (b) the firing frequency at the plateau of the response; and (c) the initial spontaneous firing frequency of the cell before any iontophoretic applications were made. Morphine was also microiontophoretically applied to single cells in each group of pretreated animals, in order to determine whether changes during chronic morphine treatment and after withdrawal were reflected by changes in the responses of single neurones to locally applied morphine.

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Results

Effects of pretreatment. Animals given sucrose alone increased in weight over the 21 days (446-503 g; $P < 0.01$, $n = 11$). Fluid intake increased sharply on days 1-3, but then dropped to a mean of 32 ml/day. Morphine-treated animals lost weight (445-409 g; $P < 0.001$, $n = 28$). Fluid intake was initially reduced but then became steady at 30-35 ml/day. Animals treated chronically with morphine and then withdrawn, lost weight rapidly during the subsequent 24 h (428-388 g; $P < 0.001$, $n = 11$). During chronic treatment the animals were often more aggressive and difficult to handle. Following withdrawal, the well documented signs of morphine abstinence, i.e. piloerection, shivering, tremor, increased vocalization during handling, increased aggression and increased defaecation, appeared.

Microiontophoretic results. Morphine-pretreated rats required more urethane (1.6-2.4 g/kg) than control animals (1.2-1.8 g/kg) for anaesthesia. On the other hand withdrawn animals appeared to be more sensitive to urethane since more fatalities occurred during induction of anaesthesia.

Analysis of firing rates. No significant differences were found (using Student's t test) in the distribution of spontaneous neuronal firing frequencies when the morphine-pretreated (295 neurones) and morphine-withdrawn (298 neurones) were compared with the control group (314 neurones).

Effects of putative neurotransmitters. The response of brain stem neurones to iontophoretically applied acetylcholine, noradrenaline and 5-hydroxytryptamine were qualitatively similar to those previously described (Bradley & Dray, 1973). No significant differences in the proportions of neurones responding to each of these three neurotransmitters was found when comparing the morphine-pretreated and -withdrawn groups with the controls (Table 1). Further, an analysis (using Student's t test) of the total number of spikes in the response to a 20 s application at 20 nA of each of the three neurotransmitters, or in the firing frequency at the plateau response, showed no significant difference between the two experimental groups, compared with their controls.

Response to microiontophoretically applied morphine. The responses were qualitatively similar in each of the three groups and both excitation and inhibition of neuronal activity, similar to that reported previously, was observed

Table 1 Proportions (% of total) of responses to microiontophoretically applied acetylcholine, noradrenaline and 5-hydroxytryptamine in control rats compared with morphine pretreated and morphine withdrawn animals

	Acetylcholine			*Noradrenaline*			*5-Hydroxytryptamine*		
	+	○	−	+	○	−	+	○	−
Control	88.4 (283)	6.9 (22)	4.7 (15)	54.9 (163)	23.2 (69)	21.9 (65)	53.8 (155)	30.6 (88)	15.6 (45)
Morphine treated	92.7 (280)	4.6 (14)	2.6 (8)	54.6 (153)	25.0 (70)	20.4 (57)	56.7 (148)	28.7 (75)	14.6 (38)
Morphine withdrawn ($n = 8$)	90.6 (271)	6.0 (18)	3.3 (10)	48.6 (137)	30.1 (185)	21.3 (60)	53.4 (143)	30.6 (82)	16.0 (43)

+, excitation; −, inhibition; ○, no effect.
The figures in parentheses refer to the number of neurones studied. There were no significant differences (χ^2 test) in any of the groups compared with controls.

(Bradley & Dray, 1974). Significantly fewer ($P < 0.01$) neurones were excited by morphine (31/67 control : 11/58 pretreated) in the morphine-pretreated group and more neurones were unaffected. However, there was no difference in the number of neurones inhibited by morphine (12/67 control : 8/58 pretreated), nor were there any differences in the numbers of neurones affected by morphine in the morphine-withdrawn group (25/62 excited : 11/62 inhibited) as compared with controls.

Whilst tachyphylaxis was observed in 12 out of 15 neurones excited by morphine but not in six neurones inhibited by morphine in the control group, tachyphylaxis to morphine excitation was not seen as frequently in the morphine-pretreated and -withdrawn animals.

Discussion Implantation, or repeated injections of morphine, have been the most commonly used methods to induce tolerance and physical dependence in animals. The experimental results reported here demonstrate that rats allowed to drink a sucrose solution containing morphine *ad libitum* for a prolonged period develop symptoms of tolerance and show morphine abstinence symptoms on withdrawal. The fact that no significant changes were found in the responses of single neurones to acetylcholine, noradrenaline and 5-hydroxytryptamine in terms of the total number of spikes occurring in a response, or in the frequency of firing at the plateau of the response, in rats showing physical dependence to morphine, or in withdrawn animals argues against the possibility that induction or unmasking of receptors occurs during development of tolerance and after withdrawal, at least as far as the three neurotransmitters studied are concerned. Furthermore, the lack of changes in the spontaneous neuronal firing rates following pretreatment with morphine and its withdrawal, implies an absence of effects on endogenous transmitter release as has been postulated.

Whilst many explanations could be offered to account for these negative results, the possibility must be considered that the method of pretreatment may not have been aggressive enough to produce neuronal changes of sufficient magnitude to be detected by the techniques used. Thus, safety factors related to transmitter synthesis, release and inactivation may be such that homeostasis is rapidly restored during chronic morphine pretreatment and after withdrawal under the conditions used.

Although it is generally considered that tolerance is associated with depressant effects of morphine, the present results indicate that some of the neuronal changes which take place during chronic morphine pretreatment, relate to morphine excitation rather than inhibition. Furthermore, these changes associated with chronic treatment may be related to the changes occurring during tachyphylaxis to morphine excitation which has been found with repeated microiontophoretic applications of morphine.

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