Morphine-induced hyperactivity in rats — a rebound effect?

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Summary. The behavioural nature of the delayed hyperactivity induced by systemic administration of morphine was studied in rats. Different components of motility induced by morphine with or without naloxone or haloperidol at different times were analyzed by observation and quantified by an Opto-Varimex-3 Activity Meter. By this automatic recording system motility was discriminated into horizontal and two different vertical components and the total distance run by each of the rats was quantified by a computer program. Simultaneously the running pattern was recorded by a XY-plotter. By means of these recordings, three subsequent phases of behaviour could be recorded after morphine (15 mg/kg i.p.): 1. a depressed phase (akinesia) lasting 1.5–2 h, followed, 2. by an intermediate phase for 1–1.5 h, still dominated by akinesia but interrupted by sudden bursts of hyperactivity. Finally, 3. a hyperactivity phase lasted for 1.5–2 h, characterized by an equal enhancement of locomotor activity and stereotypy. After 30 mg/kg of morphine the hyperactivity was predominantly characterized by locomotor activity and stereotypy and rearing were less prominent than after the smaller dose. Naloxone (2 mg/kg i.p.) given at the beginning of the hyperactivity phase significantly antagonized rearing but not other motility parameters. However, coadministration of naloxone (2 mg/kg i.p.) simultaneously with morphine (15 mg/kg) clearly antagonized akinesia and completely prevented the development of the delayed hyperactivity. Haloperidol (0.2 mg/kg i.p.) at the beginning of the hyperactivity phase clearly antagonized all of the motility parameters seen during this phase.

We conclude 1. that stereotypy and locomotor activity of the hyperactivity phase produced by morphine are not strictly combined since they follow different dose dependencies: locomotor activity increased more after the higher dose of morphine, however, stereotypy and rearing did not. 2. Akinesia and hyperactivity phase might both be triggered by an initial activation of opioid receptors immediately after injection of morphine. However, the hyperactivity phase (being dopamine dependent in all components measured) seems to develop to an opioid receptor independent process as during the hyperactivity phase it can be reversed by haloperidol but not by naloxone anymore.

Key words: Morphine — Naloxone — Haloperidol — Dopamine — Motor activity

Introduction

Systemic administration of morphine induces different patterns of behaviour in rats: small doses (1–3 mg/kg) induce an immediate locomotor stimulation (Gunné 1963; Fog 1970; Babbini and Davis 1972). Larger doses (10–40 mg/kg) induce biphasic behaviour, starting with a depressed phase and followed by a hyperactive one (Joel and Ettinger 1926; Sloane et al. 1962; Babbini and Davis 1972).

These studies were performed by observation or by more gross methods of motility registration and did not investigate the antagonism of naloxone on the biphasic behavioural pattern. From previous studies it is well known that the first, the depressed phase consists of at least two different components, rigidity, akinesia (Havemann et al. 1981) and catalepsy which is a pronounced form of akinesia (Winkler et al. 1982). The antagonism by naloxone of these different single components of the depressed phase after systemic administration of morphine has been demonstrated for the muscular rigidity (Wand et al. 1973) and for the catalepsy (Kuschinsky and Hornykiewicz 1972).

Activation of opioid receptors in the striatum seems to be important for the development of morphine-induced muscular rigidity, whereas the akinesia and catalepsy seem to result from activation of opioid receptors in the nucleus accumbens (Dill and Costa 1977; Have-
mann and Kuschinsky 1982). Furthermore, morphine enhances the dopamine turnover in the striatum (Kuschinsky and Hornykiewicz 1972; Westerink and Korf 1976) via stimulation of opioid receptors located in the substantia nigra pars compacta (Genc et al. 1983) and in the nucleus accumbens (Westerink and Korf 1976; Molemann et al. 1984), probably via stimulation of opioid receptors in the ventral tegmental area (Genc et al. 1983). This enhancement of dopaminergic activity in the striatum and in the nucleus accumbens has been previously suggested by several authors to be responsible for the development of the second phase, the hyperactivity, following the depressed phase (Joyce and Iversen 1979; Havemann and Kuschinsky 1982). The experiments of this study were performed to further investigate 1. whether all components of the delayed hyperactivity seen after systemic administration of morphine are mediated via stimulation of opioid receptors, 2. at which time activation of opioid receptors may induce hyperactivity, and 3. whether dopaminergic mechanisms contribute to the development of all components of the morphine induced hyperactivity, since several studies on opioid induced hyperactivity show the hyperactivity to be partially dopamine dependent and partially not (Havemann et al. 1985; Kalivas and Bronson 1985; Vaccarino et al. 1986).

Therefore, different single motility components after system injection of morphine were analyzed by observation and quantified by an Opto-Varimex-3 Activity Meter as recently described by Havemann et al. (1986). By this procedure, motility was differentiated into horizontal and two vertical components and the distance ran by the animal was quantified. Simultaneously the running pattern was recorded by a XY plotter and the animals were observed carefully. The motility changes were recorded after morphine alone (15 and 30 mg/kg i.p.) and after naloxone (2 mg/kg i.p.) or haloperidol (0.1 and 0.2 mg/kg i.p.) administered either simultaneously with morphine or at the beginning of the morphine-induced hyperactivity phase.

Materials and methods

Male albino Wistar rats (TNO/W 70 of F. Winkelmann, Borchen, FRG) weighing 220–240 g were used. The experiments were performed between 9 a.m. and 4 p.m. The automated recording of motility was performed by using an Opto-Varimex-3 Activity Meter of Columbus Instruments, Columbus, Ohio, USA as described by Havemann et al. (1986). Activity was recorded in an open Plexiglas cage (40 x 40 x 20 cm), the bottom of which had a sheet of paper covered with chaff and faeces. The motility was discriminated by infrared light beams into one horizontal and two vertical components. Horizontal movements were recorded by sensors 3 cm above the floor, vertical movements by sensors 10.5 cm ("vertical 1") and 16 cm ("vertical 2") above the floor. The distance between two light beams were 3 cm.

Simultaneously, horizontal movements were recorded by using a XY plotter. The Opto-Varimex-3 Activity Meter was connected via an interface to an Apple-II-europlus computer registering and storing all data separately in relation to time. In addition, a computer program written by O. Kurre and U. Havemann, calculated the counts of the horizontal and both vertical actions and the distance run by the animals.

In a previous study (Havemann et al. 1986) stereotyped behaviour was scored by observation according to the definition of oral stereotypies by Lewis et al. (1985) and simultaneously the motility was recorded in the Opto-Varimex-3 Activity Meter. By this procedure, the variables measured were defined as follows:

- **Horizontal activity**: (sensors 3 cm above floor): locomotor activity plus non-locomotor activity (e.g. head movements, oral stereotypes e.g. sniffing, licking and gnawing).
- **Vertical 1 activity**: (sensors 10.5 cm above floor): predominantly stereotyped activity, especially sniffing, but also licking and gnawing.
- **Vertical 2 activity**: (sensors 16 cm above floor): rearing and climbing movements.

**Total distance**: distance run by the animal, representing the locomotor part of motility (running activity).

The recording periods lasted for 10 min each. Two recordings were performed (40–30 min and 10–0 min) before the first intraperitoneal injection to habituate the animals to the activity meter. After the first injection the animals were kept in their home cages for 20 min until the next 10 min recording period began. The rats were placed into the recording apparatus immediately before each registration period and were always kept in their home cages between the recording periods. We used this experimental design, since usually sedatory effects, e.g. the expected effect of naloxone on morphine induced hyperactivity, are more pronounced in presence of external stimuli (e.g. handling). During all recording periods the rats were carefully observed. The experimenter was "blind" in all experiments.

All experiments of this study were performed to the same schedule: After the second habituation period the animals received the first intraperitoneal injection (morphine 15 or 30 mg/kg or 15 mg/kg morphine + 2 mg/kg naloxone, or saline), and were tested during five 10 min periods at 20 min intervals. The second intraperitoneal injection was given 150 min after the first one (either 2 mg/kg haloperidol or saline). Recording periods were continued until 330 min after the first injection. Statistical analysis. The quantified recordings in the activity meter (the horizontal and vertical activities and the total distance) are presented as median values. Saline- and morphine-treated rats were compared by Mann-Whitney U-tests at each time point both before and after the second injection.

**Drugs.** Morphine hydrochloride (Merck, Darmstadt, FRG) and naloxone hydrochloride (kindly donated by Endo Labs Inc., Garden City, N.Y., USA) were dissolved in 0.9% NaCl solution (saline) immediately before each experiment. Haloperidol (kindly donated by Janssen Pharmaceutica, Beerse, Belgium) was dissolved in 0.1% lactic acid. Doses are expressed as the salt.

**Results**

**Analysis and quantification of the motility after morphine**

Morphine (15 mg/kg i.p.) induced different profiles of motility over 5.5 h: at first a marked akinesia developed lasting 1.5–2 h. The rats showed very small movements only, which usually resulted from the handling of the animal when put into the recording cage. Then the rats sat still for the rest of the registration interval. In contrast, the saline treated rats ran along all four walls of the cage showing exploring, sniffing in the corners and rearing at the edge of the cage. They occasionally showed grooming and finally sat down to rest. This exploratory activity of saline treated rats decreased with every session.

The quantified data (Fig. 1A–D) show that morphine significantly decreased the total distance, the horizontal and vertical-1-activity. The vertical-2-activity was not significantly altered. The akinesia lasted for 1.5–2 h and was followed by a second phase with activity counts and a XY-plotter pattern similar to those in the saline controls. However, the behaviour was totally different.