Do persistent morphine effects involve interactions with the genome?

Peter S. Eriksson*, Lars Rönnbäck* and Elisabeth Hansson*

*Institute of Neurobiology and *Department of Neurology, University of Göteborg (Sweden)

(Received January 19th, 1989)

Male rats were administered morphine in a liquid diet until five days prior to mating with drug naive nulliparous female rats which received no treatment during gestation. The birth weight of resulting litters was significantly reduced. The preconceptionally morphine-treated offspring showed a 34.8% ± 17.1% mortality during the first 8 days compared with 0% in the control group and their weight gain profile was decreased as compared with controls. A persistent effect of paternally administered morphine was seen in 90-day old male offspring. A possible way to further enlighten the underlying mechanisms is proposed.

Key words: morphine; pre-conceptional exposure; genome; rat

Introduction

Pre- and early postnatal morphine administration elicits changes in the sensitivity to opiates in neonates and adult animals. It is difficult to evaluate neurochemical correlates to persistent effects per se as morphine administration elicits a cascade of cellular and intercellular events where it is impossible to determine what effects are primarily induced by morphine and what effects are induced by the interaction of morphine with other (endogenous) substances. Furthermore, the central nervous system exhibits a considerable heterogeneity as to its cellular organisation and metabolic events thereby creating methodological problems. We used the analgesia paradigm in order to focus on an anatomically defined system known to partly involve mu-receptors. Several effects of paternally administered opiates are known, such as increased neonatal mortality [1], decreased neonatal weight [2] and behavioural effects [3]. The aim of this study was: to reproduce earlier findings concerning offspring mortality and weight gain; to investigate long-lasting effects on the analgesic action of opiates; and to propose a strategy to elucidate underlying neurochemical mechanisms including genetic adjustments.

Materials and Methods

Male Sprague—Dawley rats, with an initial body weight of 300 g, and nulliparous, drug-naïve female rats, with an initial bodyweight of 270 g (obtained from A-lab, Stockholm, Sweden) were housed individually in cages (41 × 25 × 15 cm³) at a constant room temperature; lights on between 0700 and 1700 h.

Liquid diet (90 ml) was mixed with vitamins according to Zeuchner et al. [4] to a stabilized solution in a Warren Blender mixer and was administered to each rat every 24 h period in graded Richter tubes. Consumption was determined daily between 1600 and 1700 h, as was the weight of the animals. Pre-feeding on liquid diet was performed for two days to adapt the male animals to the diet prior to morphine ingestion.
Statistical analysis was according to Student's t-test. Analysis of mortality according to the Mann-Whitney test.

Analgesia was measured with the hot-plate method of Eddy and Leimbach [5]. Licking of the fore or hind paws or attempts to jump were used as end points when determining response latency. In order to establish a control response latency, each subject received two test trials, 30 min before and immediately after injection. The temperature of the hot-plate surface was maintained at 52.0 ± 0.1°C. Animals that failed to react within 60 s were assigned a 60-s-response latency.

Five male rats were treated with escalating morphine doses during an 8-day period, with an end dose of 340 mg/kg body wt/day which was maintained for two days. A five-day drug free period was allowed before mating with five nulliparous drug naive female rats. Mating was confirmed by sperm containing vaginal smears. No drugs were administered to the pregnant animals. The litters were weighed at birth and thereafter weight gain was followed until day 31. On day 90 the analgetic test was performed for controls and experimental animals, separately. Twelve animals from four different litters respectively were tested.

Results

Among litters from treated male rats birth weight was significantly reduced (Fig. 1) and mortality was 34.8% ± 17% compared with zero among controls \((P < 0.01,\) Mann-Whitney test, \(U = 0)\). No sex differences were seen concerning mortality. Mortality was evenly distributed during the first 8 days; thereafter no mortality was recorded. The weight gain was significantly decreased from day 4 to 16 as compared with controls (Fig. 2). No statistical difference in body weight was noted on day 90. The analgesic response to morphine on day 90 was markedly increased in male litters as compared to controls, resulting in significantly \((P < 0.01)\) longer response latencies in the experimental animals (Fig. 3).

Discussion

Male-mediated morphine effects on progeny is a special issue. In line with others we found that these effects consisted of a decreased weight gain neonatally [2], and an increased mortality among litters from treated males compared with controls [1]. The progeny of treated males showed an increased response to

**Birthweights**

![Birthweights](image)

**Fig. 1.** Neonatal birthweight of 63 paternally pretreated pups from five litters, compared with 47 untreated control pups from four litters. Statistical analysis were performed using Student's t-test. S.E.M. values are given. ★★ ★★ \(P < 0.001\).
the analgesic action of morphine. It has been proposed that these effects could be due to drug-induced genetic defects, though this is unlikely, since the F2 generation has been shown to be unaffected [6]. Morphine is known to interact preferentially with the μ-receptor.

The morphine receptor is generally considered to be connected to the cyclic-AMP second messenger system.

Stimulation of the μ-receptor results in a decreased accumulation of cyclic-AMP [7,8]. Alterations in the intracellular concentration of

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Fig. 2. The weight gain of pups from five different litters of the preconceptionally treated group is compared to the weight gain of the control animals of four litters. Statistical analysis were performed using Student's t-test. S.E.M. values are given. ★★P < 0.01.

Fig. 3. Hot plate latency vs time is presented in relation to an i.p. injection of 7.5 mg/kg body wt. morphine indicated by the vertical arrow. Twelve male animals from four experimental litters were selected randomly, as were twelve male control animals. Areas under respective curve were calculated. Statistical analysis was performed using Student's t-test. S.E.M. values are given. ★★P < 0.01.
calcium has also been reported as a result of opiate receptor stimulation, [9] which could indicate interactions with calmodulin, protein kinase C or both [10]. A second messenger effect, besides the activation of different protein kinases, has recently been indicated in terms of a direct genetic response. Brain-specific genetic components, so-called identifiers, have been isolated [11]. These components have been implicated to be involved in the regulation of the transcription of brain-specific genes. Furthermore, the connection between second messengers and inducible DNA alterations has been shown both for the cyclic AMP system and the phorbol esters [12]. Opioid gene expression has been implicated to be modulated via opioid receptors [13]. Several examples of inducible genetic alterations exist within the phyllogenetic hierarchy. Some viruses invert DNA segments in order to adjust to different hosts [14]. Some bacteria utilize movable genetic elements that could be transposed from one position to another. Transposable elements can contain regulatory signals in control of neighboring genes [15]. Some lower eucaryotes have arrays of silent genes which are not expressed. An auxiliary gene copy can be synthesized of any of them, and then inserted into a particular site on the chromosome where it can be read. An auxiliary copy can substitute a gene of expression and thus change the phenotypic expression of the cell [16]. With the amount of DNA corresponding to 100 antibody genes, B-lymphocytes are able to face the need of making tens of millions of types of antibody molecules, by splitting the antibody genes into pieces and then splicing these pieces together in a different way [17]. The presence of gene amplification [18] i.e. the transduction of hundreds of copies of an active gene, or a mechanism related to some of the above mentioned are not unlikely, considering the infinitely more complex machinery governing the cells of mammals.

The existence of related mechanisms within the CNS could possibly be of significance for the induction of tolerance to drugs. There are several plausible alterations responsible for tolerance to morphine. Receptor density could be modified. Furthermore, the second messenger systems connected to the endogenous opiate receptors could be modified, e.g., on the level of the GTP binding protein in the cAMP system, or modifications in the calcium-binding properties intracellularly (altered calmodulin levels).

The release and processing of endogenous opiate precursors could be modified, as well as the degrading enzyme systems. However even if tolerance is considered to last for days and even weeks and furthermore not being accompanied by rebound phenomena, it has been difficult to separate manifestations of it from adaptational alterations. By paternal preconceptional morphine treatment, possibly inducing alterations within the cells in the spermatogenesis, pure manifestations of transgenerational drug-induced effects in the CNS of the progeny could be detected. Furthermore, specific effects on receptors and second messengers and their mechanisms on the cellular level could possibly be elucidated, using primary cultures of both neurons and glial cells derived from the offspring.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council (25X-06005), Torsten and Ragnar Söderbergs's Foundation, Stiftelsen Anna Ahrens's Foundation, Anders Otto Swärd's Foundation and the Medical Faculty, University of Göteborg.

References