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Effects of opioid agonists on urine production in neonatal rats

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Abstract—The modulatory effects of opioids on urine production in adult rats have been well-documented. We report here the first investigation of the effects of these agents on urination in neonatal rats. The κ-agonists U50,488H (1,10 mg kg^{-1}) and (+)-tifluadom (10 mg kg^{-1}) produced an increase in urine output in 10-day old pups whereas the (−)-isomer of tifluadom was ineffective in this model. The diuretic effects of the highest dose of U50,488H were attenuated by a 10 but not a 1 mg kg^{-1} dose of the opioid antagonist naltrexone. These findings suggest that κ-agonists, as in adult animals, produce diuresis in neonates by activity at κ-opioid receptors and also confirm the stereoselective nature of the response. The increase in urination produced by U50,488H (10 mg kg^{-1}) was also reduced by the α-adrenoceptor antagonist phentolamine (1 mg kg^{-1}), an observation which supports the hypothesis that κ-agonists—in addition to their well-established inhibitory effects on the release of antidiuretic hormone—may increase urination via an adrenergic mechanism at the level of the adrenal medulla. The μ-opioid agonist morphine (0·1-10 mg kg^{-1}), in contrast to its observed effects in older animals, did not produce antidiuresis in either normally-hydrated or water-loaded 10-day old rat pups. The results of this study therefore show that the stimulatory effects of κ-agonists on urine production appear to be fully-functional at 10-days but the inhibitory effects of opioids on urination lag behind in development.

Opioid agonists have been reported to produce various effects on urination in adult rats. For instance, κ-agonists induce marked diuresis and it has been suggested that this increase in urination may be used as a simple in-vivo test for κ-activity (Leander 1983a,b). In comparison, μ-opioid agonists have been shown to inhibit urine output in fully-grown rats (De Bodo 1944; Huidobro 1978). We have previously reported that κ- and μ-opioid agonists produce different behavioural profiles in neonatal and adult animals (Jackson & Kitchen 1989a). Hence, in the current study we have investigated the effects of these compounds on urine production in 10-day old rat pups using the selective κ-agonists U50,488H (Von Voigtlander et al 1983) and tifluadom (Römer et al 1982a,b) and the classical μ-opioid morphine. In addition, the mechanisms underlying the effects of opioids on urine production in neonates have been investigated using the opioid antagonist naltrexone and the α-adrenoceptor antagonist phentolamine.

Materials and methods

Animals. Experiments were performed on Wistar albino rats. All animals were maintained at a temperature of 21 ± 1°C under a 12h light-dark cycle (lights off at 19.00h) with continuous access to normal rat diet and tap water. Male and female rats from the University of Surrey breeding stock were mated and the females were rehoused in individual cages on the confirmation of pregnancy. On the day of birth (postnatal day 0) litters were standardized to 8 pups per dam, each litter containing approximately equal numbers of each sex. Rat pups then remained with the mother in the home cage until required for use on postnatal day 10.

Measurement of urine output. Urine output in 10-day old rats was measured by a method (adapted from Kavlock & Gray 1982) which takes into account the fact that neonatal rats do not normally micturate spontaneously. Instead, urination in these animals is largely controlled by the mother who stimulates urine flow by licking the anogenital region of the pup.

On the day of test mothers and pups were removed to a quiet laboratory and allowed to acclimatize for 1 h at an ambient temperature of 25°C before experimentation began. All procedures were carried out in the light phase between 10.00 h and 16.00 h. Pups were separated from the dam and their bladders were voided by gently stroking the perianal region with a small rod to mimic the mothers grooming behaviour. Pups were then weighed to the nearest 0·01 g. Litters were divided into four treatment groups and after drug administration pups were placed individually in 2 litre beakers which contained paper towel bedding (to aid detection of any urine or faeces excreted during the experiment). At the end of a 2 h period pups were reweighed both before and after their bladders were voided. The neonatal rats did not have access to any fluid throughout the trial and there were no overt effects of any of the test compounds on the negligible amount of faeces expelled by the pups during the experiment. Accordingly, the changes in body weight determined 2 h after drug administration were taken as a measure of either spontaneous or stimulated urine flow. All 8 animals in each litter were tested concurrently and drug treatment groups contained 6 pups which were obtained from at least 3 different litters.

Drugs. The drugs used were: U50,488H (trans-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methane sulphonate hydrate, gift from Upjohn); (+)- and (−)-tifluadom (1-methyl-2(3-thienylcarbonyl)-aminomethyl-5-(2-fluorophenyl)-H-2,3-dihydro-1,4-benzodiazepine, gifts from Kali-Chemie AG); morphine sulphate (May & Baker); naltrexone hydrochloride (courtesy of Du Pont Pharmaceuticals) and phentolamine mesylate (Ciba-Geigy). U50,488H, morphine and naltrexone were dissolved in sterile 0·9% saline and the isomers of tifluadom were dissolved in 20% ethanol. All drugs were administered intraperitoneally in a dose volume of 0·1 mL/20g body weight and in the interaction studies naltrexone or phenotolamine were administered concurrently with U50,488H.

Statistical analysis. Results are expressed in terms of the Diuretic Index (% change in initial body weight). Treatment group means ± s.e. mean were calculated and statistically compared using analysis of variance and the Dunnett's test.

Results

Initial body weights of the 10-day old pups were in the range 20–30g and there were no significant differences in mean body weights of the drug treatment groups at the start of each experiment. Stimulation of the bladders of the pups immediately after removal from the dam did not normally result in any expulsion of urine and control animals exhibited only a very low level of micturation during the 2 h test.

Administration of the κ-agonist U50,488H (1, 10 mg kg^{-1} i.p.) produced overt signs of both spontaneous and stimulated urination in 10-day old rat pups. These diuretic effects resulted in significant decreases in body weight which were particularly evident after the pups bladders had been stimulated manually

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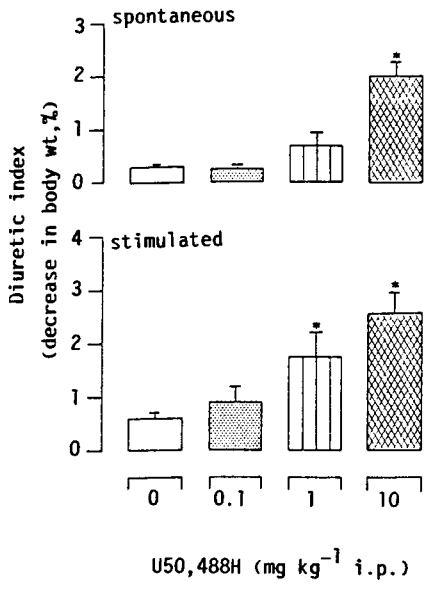

FIG. 1. Effects of U50,488H (0·1–10 mg kg^{-1} i.p.) on spontaneous and stimulated urination (measured in terms of the Diuretic Index- % decrease in body weight) in 10-day old rat pups. Values represent mean scores ± s.e. mean for groups of 6 animals. Significant differences from the vehicle-treated controls are denoted by * $P<0.05$.

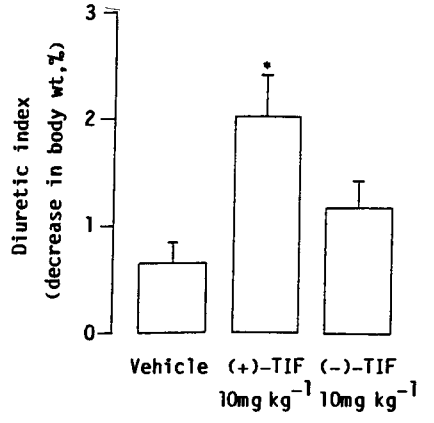

FIG. 2. Effects of (+)- and (−)-tifluadom (10 mg kg^{-1} i.p.) on stimulated urination (measured in terms of the Diuretic Index- % decrease in body weight) in 10-day old rat pups. Values represent mean scores ± s.e. mean for groups of 6 animals. Significant differences from the vehicle-treated controls are indicated by * $P<0.05$.

(Fig. 1.) (+)-Tifluadom (10 mg kg^{-1})—but not a corresponding dose of its (−)-enantiomer—also produced diuresis with an associated drop in body weight in 10-day old rats after bladders had been voided (Fig. 2).

The effects of U50,488H (10 mg kg^{-1}) on stimulated urination in the neonatal rats were reversed by concurrent administration of 10 but not 1 mg kg^{-1} naltrexone (Fig. 3) and also by administration of the α-adrenoceptor antagonist phentolamine (1 mg kg^{-1}; Fig. 4). The doses of naltrexone and phentolamine used in this study had no effects on urine output in the 10-day old pups (after bladders had been artificially voided).

The μ-agonist morphine (0·1–10 mg kg^{-1}) had no effects on either spontaneous or stimulated urine production in normally-hydrated neonatal rats (data not shown), nor did this compound alter the increased urination which occurred after pups were given a water load (0·5 mL p.o., 2–3% initial body weight) at the beginning of the experiment (e.g. Diuretic Index for control

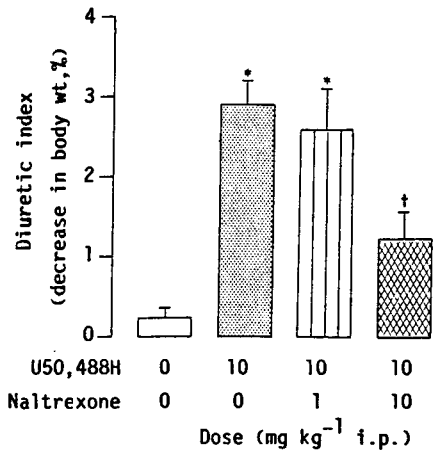

FIG. 3. Effects of naltrexone (1, 10 mg kg^{-1} i.p.) on the increase in stimulated urination induced by U50,488H (10 mg kg^{-1} i.p.) in 10-day old rat pups. Values represent mean scores ± s.e. mean for groups of 6 animals. Significant differences from the vehicle-treated controls are shown by * $P<0.05$ and from the drug-treated controls by † $P<0.05$.

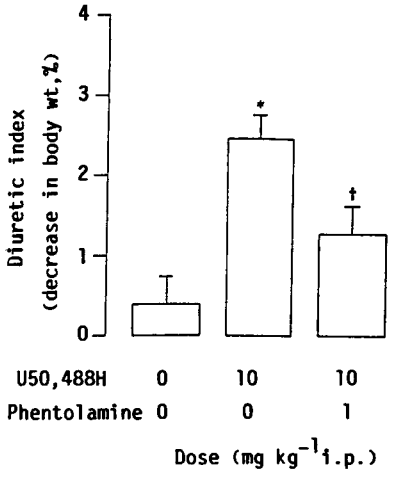

FIG. 4. Effects of phentolamine (1 mg kg^{-1} i.p.) on the increase in stimulated urination induced by U50,488H (10 mg kg^{-1} i.p.) in 10-day old rat pups. Values represent mean scores ± s.e. mean for groups of 6 animals. Significant differences from the vehicle-treated controls are denoted by * $P<0.05$ and from the drug-treated controls by † $P<0.05$.

animals −0·8 ± 0·1, rats treated with morphine (10 mg kg^{-1}) following spontaneous urination −0·6 ± 0·1; stimulated controls −1·8 ± 0·4, rats treated with morphine (10 mg kg^{-1}) following manual stimulation −1·1 ± 0·2).

Discussion

The κ-agonist U50,488H increased both spontaneous and stimulated urination in 10-day old rat pups. This response was comparable to the diuretic effects of corresponding doses of U50,488H in adult animals (Leander 1983b). The increased urination observed after treatment with (+)-tifluadom—but not its (−)-enantiomer—corroborates the involvement of κ-receptors in opioid-induced diuresis in neonates and confirms its stereoselective nature. Similar findings have been reported in fully-grown rats (Shearman & Tolcsvai 1986). Kappa-induced diuresis in the neonates was reversed by naltrexone, hence reflecting an interaction with opioid receptors. The relatively high dose of antagonist (10 mg kg^{-1}) required to attenuate this parameter accords with the high doses of opioid antagonist

required to inhibit κ-diuresis in older animals (Leander 1983a,b). Moreover, as κ-induced hyperactivity in developing rats was abolished by a lower dose of 1 mg kg^{-1} naltrexone (Jackson & Kitchen 1989a), the decrease in body weight observed in 10-day old pups after treatment with U50,488H cannot be explained simply in terms of a generalized increase in activity and metabolic rate.

There is substantial evidence that κ-diuresis in adults involves a central component, thus, intracerebroventricular administration of U50,488H produces an increase in urine flow (Cowan & Khunawat 1986). Furthermore, the diuretic effects of κ-agonists are not reduced by quaternary analogues of naloxone which do not readily penetrate the blood-brain barrier (Leander 1983a; Shearman & Tolcsvai 1986). It is difficult to assess whether κ-diuresis in neonates is under the control of central sites due to the immaturity of the blood-brain barrier until the third postnatal week (Himwich 1962). However, it is relevant to note that κ-receptors (and also μ-receptors) are present in the rat central nervous system at birth (Spain et al 1985) and hence could be functional in regulating water balance from an early age. Moreover, κ-induced diuresis appears to be mediated, at least in part, by inhibition of the release of antidiuretic hormone from the neurohypophysis (Miller 1975; Iversen et al 1980; Leander 1983a,b; Leander et al 1985). This hormone has been shown to reduce the diuretic effects of water-loading in one-day old rat pups (Kavlock & Gray 1982) also suggesting that the mechanisms underlying κ-diuresis are present by postnatal day 10.

In addition to a central site of action, a peripheral component has recently been implicated in κ-diuresis, hence, κ-agonists do not increase urine flow in rats following bilateral adrenal demedullation (Blackburn et al 1986). These authors investigated the effects of α-adrenoceptor antagonists on κ-induced diuresis and concluded from the lack of inhibition that adrenal catecholamines are not involved in the stimulatory effects of opioids on urine flow. However, others have reported that κ-induced urination may be reversed by coadministration of phentolamine (a non-selective α-antagonist) and idazoxan (the selective α_2-antagonist) and hypothesized that κ-agonists may induce release of catecholamines from the adrenal medulla and that the circulating catecholamines may then activate α_2-adrenoceptors to produce diuresis (Birch & Hayes 1988). This concept is supported by observations that α_2-receptor agonists modulate urine output in adult rats (Gellai & Ruffolo 1987; Birch & Hayes 1988) and also by the current findings that the diuretic effects of U50,488H are reduced by phentolamine in a corresponding manner in young animals.

Finally, the μ-agonist morphine failed to produce antidiuresis in either normally-hydrated or water-loaded 10-day old rat pups (which exhibited a diuretic response to the oral water load). In comparison, clear antinociceptive effects of μ-opioid agonists have been observed at this age (Jackson & Kitchen 1989b). The mechanisms underlying μ-antidiuresis in adult rats have not been fully elucidated as yet, though, these responses appear to be independent of increases in antidiuretic hormone release and may instead arise from a centrally-mediated reduction in glomerular filtration rate (see Huidobro-Toro & Huidobro 1981; Grell et al 1985; Leander et al 1985) or from direct effects on bladder function (Dray & Metsch 1984).

In conclusion, we have shown differential development of the effects of κ- and μ-opioid agonists on urine production in the rat. Kappa-induced diuresis appears to be fully functional 10-days after birth though the antidiuretic effects of the μ-agonist morphine are not apparent in this age group—presumably reflecting delayed maturation of μ-receptors (or associated neuronal systems) in those central and/or peripheral sites responsible for mediating this response.

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