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The effects of N-(cyclopropylmethyl)-19-isopentyl-nororvinol (M320), a potent agonist at κ- and μ- opiate receptors, on urine excretion of rats

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1 The effects of N-(cyclopropylmethyl)-19-isopentylnororvinol hydrochloride (M320) on urine excretion by rats were investigated. Further studies, using rabbit isolated vas deferens, investigated its interactions with κ-opiate receptors.

2 The output of urine for a 2 h period after M320, administered subcutaneously to normally hydrated Long Evans rats, showed a bell-shaped dose-response relationship, the maximum effect occurring after 10 $\mu g\,kg^{-1}$. Urinary retention contributed to but did not fully account for the weaker diuresis after high doses. Attenuation of the ascending portion of the dose-response curve to M320 occurred after 1 and 10 $mg\,kg^{-1}$ but not 0.1 $mg\,kg^{-1}$ of naltrexone intraperitoneally.

3 M320 in low doses (3–10 $\mu g\,kg^{-1}$) caused a small but significant increase in sodium excretion. M320 (30 $\mu g\,kg^{-1}$) reduced both sodium and potassium excretion.

4 M320 (10 $\mu g\,kg^{-1}$ s.c.) did not increase the volume of urine voided in 2 h by Brattleboro rats showing diabetes insipidus, even when urine excretion was reduced to normal by 1 week of vasopressin replacement.

5 The volume of urine voided in 4 h by Brattleboro rats was progressively reduced to zero by M320 (10–100 $\mu g\,kg^{-1}$ s.c.). Urinary retention contributed to but did not account for this reduction.

6 Plasma levels of immunoreactive arginine vasopressin (ir-AVP) were reduced in both normal and dehydrated Long Evans rats after doses greater than 1 $\mu g\,kg^{-1}$ M320 s.c.

7 *In vitro*, M320 caused persistent inhibition of twitches of the electrically stimulated rabbit vas deferens (IC_{50} 1.7 nM).

8 These data suggest that M320 has potent opioid agonist activity at κ-receptors and at higher concentrations stimulates μ- receptors. In the rat, its activity on κ-receptors is associated with diuresis and suppression of plasma vasopressin levels. The antidiuresis seen after high doses may be due to its activity on μ-receptors, possibly at a central site.

Introduction

Bentley *et al.*, (1965) synthesized a series of derivatives of thebaine which yielded some of the most potent opioids known. One of these, M320 (N-(cyclopropylmethyl)-19-isopentylnororvinol HCl), was found to possess powerful antinociceptive and diuretic properties in the rat (Boura & Fitzgerald, 1966). Diuresis caused by M320 was accompanied by little or no change in sodium or potassium excretion. More recently, Evans (1983) showed that the dose-response curve for the effect of M320 on urination was bell-shaped, the drug causing no diuresis at high doses. In the present study we have investigated in more detail the effects of the drug on urine excretion of rats, endeavouring to identify the nature of the opiate receptors concerned and the mechanisms responsible. A preliminary account of some of these findings has been given to the Australian Neuroscience Society (Olley *et al.*, 1985).

Methods

Long Evans and homozygous (DI/DI) Brattleboro rats of either sex (200 - 300 g) were housed in groups of 5 with free access to food (Clark King GR2). Rats were

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used either in a state of normal hydration, with access to water *ad libitum* until 30 min before administration of drugs, or were deprived of water for 36 h before the experiment. Food and water were not available during experiments. Male mongrel rabbits (2.5–3.5 kg) were housed individually with free access to food (Clark King SG1) and water. Rooms were illuminated from 06 h 00 min to 18 h 00 min and maintained at 20°C.

Urine production by normal rats

All experiments were performed between 09 h 00 min and 14 h 00 min at an ambient temperature of 20°C. Animals were used on one occasion only. Male Long Evans rats were injected subcutaneously with saline or one of a series of doses of M320 (0.1–30 $\mu g\,kg^{-1}$), bremazocine (1–3000 $\mu g\,kg^{-1}$), ethylketocyclazocine (0.03–3 $mg\,kg^{-1}$) or ketocyclazocine (0.03–2 $mg\,kg^{-1}$) and placed immediately in individual metabolism cages. Urine output was measured every 30 min for 4 h. The sodium and potassium concentrations of each 30 min urine sample were determined ($n = 10–11$ per group, except for ketocyclazocine when $n = 5$).

Further normally hydrated male Long Evans rats were treated in groups of 5 with saline or M320 (3–100 $\mu g\,kg^{-1}$ s.c.). At the end of the 4 h period each rat was anaesthetized with pentobarbitone (100 $mg\,kg^{-1}$ i.p.). Immediately after loss of the corneal reflex the abdomen was opened and the urinary bladder content aspirated with a syringe and needle. Sodium and potassium concentrations and the volume of this residual urine were also measured.

To study the effects of naltrexone on urine excretion caused by M320, male Long Evans rats in groups of 5 were pretreated with either saline or naltrexone (0.1, 1.0 or 10.0 $mg\,kg^{-1}$ i.p.); 30 min later the animals were given either saline or M320 (1, 3, 10 or 30 $\mu g\,kg^{-1}$ s.c.) and then placed immediately in metabolism cages. Urine output was measured from 30–150 min after administration of saline or M320.

Urine production by Brattleboro rats

Male Brattleboro rats in groups of 5 were injected subcutaneously with saline, M320 (1–100 $\mu g\,kg^{-1}$) or morphine (1–10 $mg\,kg^{-1}$) and urine output collected every 30 min for 4 h. The sodium and potassium content of each urine sample was assessed. After 4 h the residual volume of urine in the bladder was measured by the procedure described above.

Further groups of 10 Brattleboro rats were treated daily with either vasopressin tannate (1 $u\,kg^{-1}$ i.m.) or vehicle (peanut oil). Normal Long Evans rats were injected with the vehicle only. On day 6, 30 min after treatment with vasopressin tannate or vehicle, rats were given either saline or 10 $\mu g\,kg^{-1}$ M320 s.c. and placed immediately in metabolism cages. Urine output during the following 2 h was monitored.

Electrolytes

Electrolyte (Na^+ and K^+) concentrations in urine samples were measured with a Corning P435 flame photometer.

Plasma vasopressin determination

Plasma immunoreactive arginine vasopressin (ir-AVP) concentrations were determined by radioimmunoassay using the method and antiserum of Woods & Johnston (1983). Antibodies to synthetic arginine-vasopressin were raised in New Zealand White rabbits after conjugating the peptide to haemolymph plasma powder by glutaraldehyde. The final dilution of antiserum that bound 50% of the tracer was 1:120,000. The antibody cross-reacted 10% with synthetic lysine-vasopressin, 100% with 1-desamino-8-D-AVP (DDAVP) and less than 0.001% with synthetic oxytocin, arginine vasotocin and human neurophysins I and II. Synthetic AVP (Ferring, AB, Malmo, Sweden) was used as standard. To obtain plasma samples, rats were decapitated and trunk blood collected into chilled heparinized tubes. After centrifugation, the plasma was separated and extracted with acetone/petroleum ether. Mean recovery of AVP was 83.2 ± 2.8%, and was linear over the range of 0–20 pg. Assay sensitivity was 0.65 $pg\,ml^{-1}$ and the intra- and interassay variabilities were 8.4% and 11.6%, respectively. Plasma ir-AVP concentrations were not corrected for extraction efficiency.

In vitro *studies*

Animals were killed by stunning and cervical dislocation before removal of tissues. The rabbit isolated prostatic vas deferens was mounted under 500 mg tension in a 10 ml organ bath containing oxygenated physiological solution at 37°C (composition, mM: NaCl 118, KCl 4.75, $CaCl_2$. $2H_2O$ 2.54, KH_2PO_4 0.93, $NaHCO_3$ 25.0 and glucose 11.0). Transmural stimulation (0.1 Hz, 0.5 ms pulse duration and supramaximal voltage) was applied via platinum electrodes from a Grass S88 stimulator. The resulting twitches were recorded using isotonic Harvard transducers (386) linked to Rikadenki (DB4) polygraphs. A single cumulative concentration-response curve was determined from each tissue ($n = 4–7$), adding the drug to the bath at 15 min intervals. After each experiment organ baths were dismantled and sonicated in 20% Extran 100 to remove M320 adsorbed to surfaces before re-use. Contaminated tubing was discarded.

Materials

The following drugs were used: N-(cyclopropylmethyl)-19-isopentylnororvinol HCl, (M320; Reckitt and Colman); ethylketocyclazocine methane sulphonate and ketocyclazocine, (Sterling Winthrop); naltrexone HCl (Endo); [Leu]enkephalin (Sigma); morphine HCl, (Macfarlan-Smith); vasopressin tannate, (Parke Davis), bremazocine HCl (Sandoz) and (−)-2-(3-furylmethyl)-2'-hydroxy-5, 9 -diethyl-6, 7-benzomorphan (Mr2266), (Boehringer Ingelheim KB). All drugs were calculated as base. For *in vivo* experiments they were dissolved in 0.9% w/v NaCl solution (saline) and administered in a volume of 1 ml kg^{-1} except for vasopressin tannate which was dissolved in peanut oil and administered in a volume of 0.4 ml kg^{-1}. For studies using isolated tissues, drugs were dissolved in distilled water and diluted with physiological fluid.

Statistical analysis

Statistical analysis of the *in vivo* experiments was performed by use of Student's *t* test. *P* values <0.05 were considered to be significant. Calculations of *in vitro* IC_{50} values were performed using the least square method of regression analysis. All *in vivo* results are given as mean ± s.e.mean. *In vitro* IC_{50} estimates are given as the means with their 95% confidence limits.

Results

Effects on urination of normal Long Evans Rats

The urine output of normally hydrated male Long Evans rats measured after subcutaneous administration of saline, M320, bremazocine, ketocyclazocine and ethylketocyclazocine is shown in Figure 1. Saline treated animals produced 2.12 ± 0.38 ml kg^{-1} urine in 2 h. The dose-response curve for M320 was bell-shaped. Urine output was significantly increased after 1.0, 3.0 or 10.0 μg kg^{-1} of M320 but not after 30 μg kg^{-1} when compared to that of saline treated animals. Ethylketocyclazocine also showed a similarly shaped dose-response relationship but was approximately 100 times less potent. Bremazocine and ketocyclazocine increased urination in a dose-related manner.

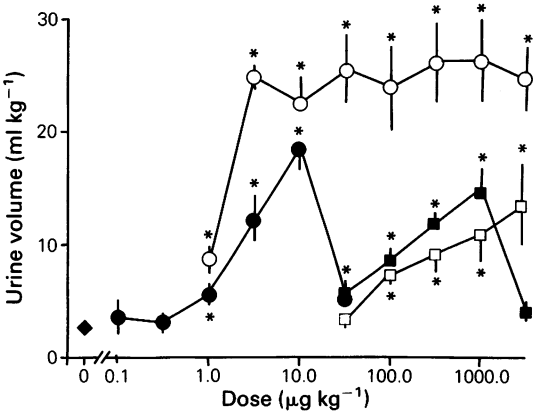

Figure 1 The effects of saline (◆), M320 (●), bremazocine (○), ketocyclazocine (□), and ethylketocyclazocine (■) on urine output of normally hydrated rats in the two hour period following subcutaneous drug administration. Each point is the mean of 10–11 observations except for ketocyclazocine when $n = 5$; a vertical line shows s.e.mean. *Significantly different from control.

Comparison of the ascending portions of the dose-response curves only, showed that equieffective doses to induce voiding of 10 ml urine kg^{-1} in 2 h were bremazocine 1.2, M320 2.1, ethylketocyclazocine 149 and ketocyclazocine 562 μg kg^{-1}.

Investigation of the bell-shaped dose-response curve for the effect of M320 on urination was attempted in further groups of normally hydrated Long Evans rats by measuring not only the volume of urine voided but also the volume of urine retained in the bladder (Table 1). These animals also gave a bell-

Table 1 Urine production by normally hydrated male Long Evans rats in the 4 h period following treatment with M320.

M320 (μg kg^{-1}, s.c.)	*Urine voided* (ml kg^{-1})	*Urine remaining in bladder* (ml kg^{-1})	*Total urine volume* (ml kg^{-1})
0	8.6 ± 1.7	2.0 ± 0.3	10.7 ± 1.6
3	19.5 ± 3.9*	1.4 ± 0.5	20.9 ± 3.5*
10	39.7 ± 4.9*	2.9 ± 0.8	42.7 ± 5.4*
30	31.4 ± 8.1*	5.7 ± 0.5*	37.1 ± 7.9*
100	6.7 ± 1.2	6.1 ± 1.4*	12.8 ± 2.6

Total urine volume is the sum of the urine voided and the urine remaining in the bladder by the end of the 4 h period. Values are the means of 5 observations ± s.e.mean. *Differs significantly from saline treated animals ($P<0.05$).

shaped dose-response relationship when considering the volume of urine voided over the 4 h period, however, diuresis was still evident after 30 but not after 100 $\mu g\,kg^{-1}$ M320 in this series. Retention of urine in the bladder was significant after 30 and 100 $\mu g\,kg^{-1}$ M320 and so contributed to but did not entirely account for the lack of diuretic activity seen after 100 $\mu g\,kg^{-1}$.

The effect of naltrexone pretreatment on the urine output after M320 was complex and depended on the dose of M320 received (Figure 2). The ascending portion of the M320 dose-response curve was significantly shifted to the right, dose-dependently, by 1.0 and 10.0 but not by 0.1 $mg\,kg^{-1}$ of naltrexone. The volume of urine voided by animals treated with 30 $\mu g\,kg^{-1}$ M320 was significantly greater when they had been pretreated with 1.0 but not with 0.1 or 10.0 $mg\,kg^{-1}$ of naltrexone.

Table 2 shows the total urinary excretion of sodium and potassium ions recorded 2 h and 4 h after M320 administration. These data show that a small but significant increase in sodium but not potassium excretion accompanied the diuretic effect of M320. A decreased excretion of both sodium and potassium occurred concomitantly with the lack of diuresis seen after 30 $\mu g\,kg^{-1}$ M320 s.c.

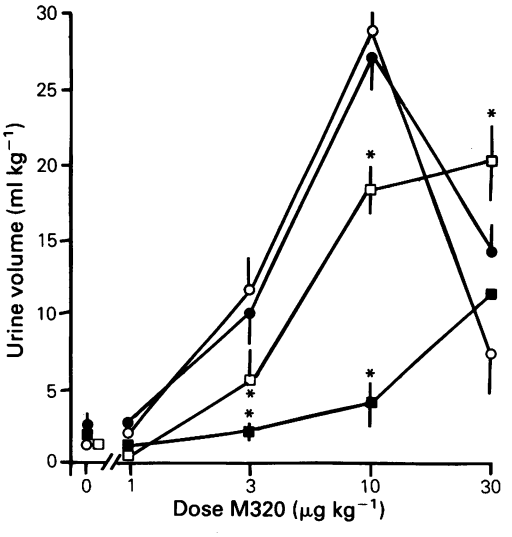

Figure 2 The effect of saline (○) or naltrexone 0.1 (●), 1.0 (□) and 10.0 (■) $mg\,kg^{-1}$, given intraperitoneally 30 min before M320 s.c., on the volume of urine voided by normally hydrated male Long Evans rats 30–150 min after the administration of M320. *Naltrexone-treated rats differ significantly from their control ($P < 0.05$).

Effects of urine excretion of Brattleboro rats

Figure 3 shows the lack of effect on Brattleboro rats of a dose of M320 which was diuretic in normal rats. The large volume of urine voided in 2 h by normally hydrated Brattleboro rats, with congenital diabetes insipidus, was reduced by vasopressin replacement therapy for 6 days from 31.9 ± 5.3 to 4.17 ± 1.4 $ml\,kg^{-1}$. This was not significantly different from the 1.5 ± 0.6 $ml\,kg^{-1}$ urine voided by normal Long Evans rats. After 10 $\mu g\,kg^{-1}$ M320 s.c. the urinary output of Long Evans rats treated with vehicle was increased to 21.5 ± 3.9 $ml\,kg^{-1}$ whereas there was no significant increase in the volume of urine voided by Brattleboro rats, irrespective of whether or not they had been given vasopressin replacement therapy, when compared to their respective controls.

The effects of M320 on the distribution of urine

Table 2 Sodium and potassium excretion of normally hydrated male Long Evans rats in the 2 h and 4 h periods after saline or M320 administration.

	Collection period			
	0–2 h		*0–4 h*	
	Electrolyte ($\mu mol\,kg^{-1}$)			
	Na^+	K^+	Na^+	K^+
Treatment				
Saline (s.c.)	33.9 ± 7.1	57.3 ± 16.9	63.2 ± 16.4	183.5 ± 64.4
M320 ($\mu g\,kg^{-1}$, s.c.)				
0.1	60.3 ± 24.4	54.2 ± 17.8	76.9 ± 26.4	147.1 ± 44.6
0.3	44.4 ± 14.0	80.2 ± 32.5	79.5 ± 23.3	172.2 ± 56.4
1.0	49.1 ± 13.4	63.3 ± 17.5	62.2 ± 16.0	96.4 ± 16.7
3.0	82.8 ± 15.3*	63.2 ± 12.5	94.8 ± 17.6	96.7 ± 13.3
10.0	86.4 ± 19.5*	94.8 ± 21.5	115.7 ± 21.5*	144.7 ± 27.2
30.0	23.5 ± 10.2	21.4 ± 5.8*	30.1 ± 10.9*	34.4 ± 9.6*

$n = 10–11$ rats per group. Na^+ and K^+ excretion ($\mu mol\,kg^{-1}$) expressed as mean ± s.e.mean. *Differs significantly from control ($P < 0.05$).

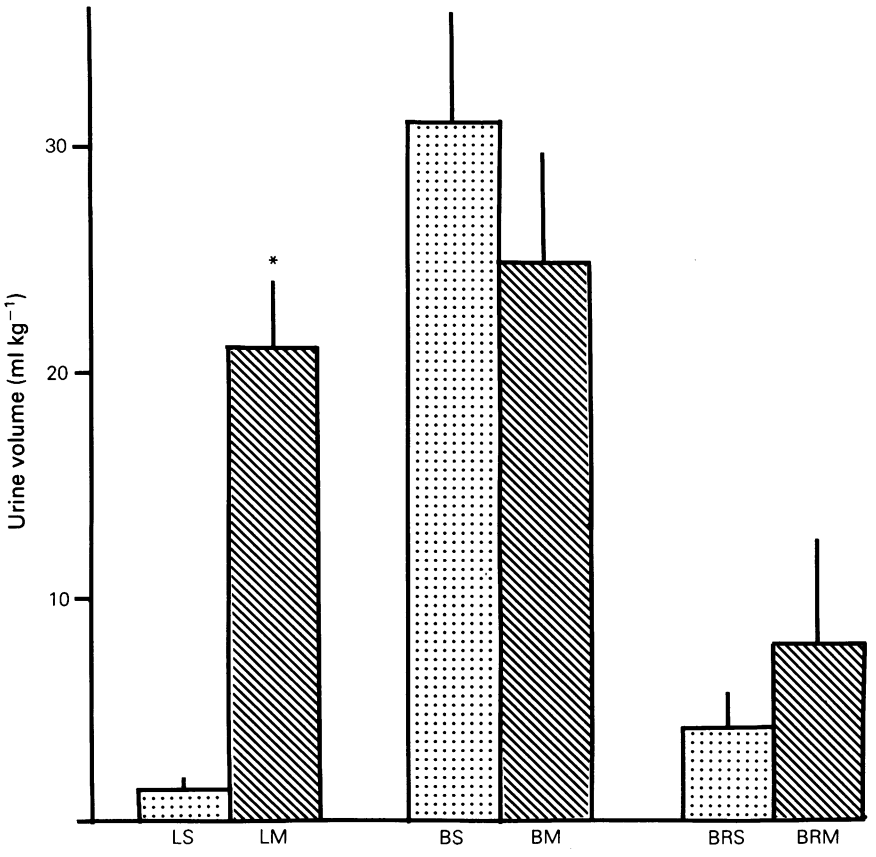

Figure 3 Volume of urine voided 0–2 h after subcutaneous saline (S, stippled columns) or M320 10 μg kg^{-1} (M, hatched columns) by normally hydrated Long Evans (L) and Brattleboro (B) rats with (R) and without AVP replacement therapy (1 u kg^{-1} daily for 6 days). Values are the means from 10 rats; a vertical line shows s.e.mean. *M320-treated animals differ significantly from their controls ($P < 0.05$).

voided and retained in the bladders of Brattleboro rats are shown in Figure 4. Normally hydrated Brattleboro rats formed large volumes of urine in 4 h, of which 43.8 ± 3.1 ml kg^{-1} was voided and 6.8 ± 0.9 ml kg^{-1} retained. Rats treated with M320 (10–100 μg kg^{-1}) or morphine (3 and 10 mg kg^{-1}) voided significantly smaller volumes during the same period but greater volumes in the bladder were retained after M320 (3.0–100.0 μg kg^{-1}). Total volumes of urine formed were not increased after any dose of either drug and were significantly decreased, dose-dependently, after the higher doses of M320 (30 and 100 μg kg^{-1}) and morphine (3 and 10 mg kg^{-1}).

Effect on plasma levels of ir-AVP in normally hydrated and dehydrated Long Evans rats

Table 3 shows the plasma ir-AVP concentration of normally hydrated Long Evans rats which was 2.38 ± 0.61 pg ml^{-1}. In comparable rats ($n = 24$), 90 min after treatment with M320 (1–300 μg kg^{-1} s.c.), the plasma ir-AVP content of every animal was found to be near or below the assay detection threshold of 0.65 pg ml^{-1}. The plasma ir-AVP of rats deprived of water for 36 h was significantly elevated, when compared to hydrated animals, and this high level was found to be dose-dependently reduced 90 min after administration of 3 and 30 μg kg^{-1} of M320 s.c.

Effects in vitro

Investigation of the effects of M320 on isolated preparations was complicated by autoinhibition. This was caused partially by its biological properties and partly by it leaching out into the bath fluid after

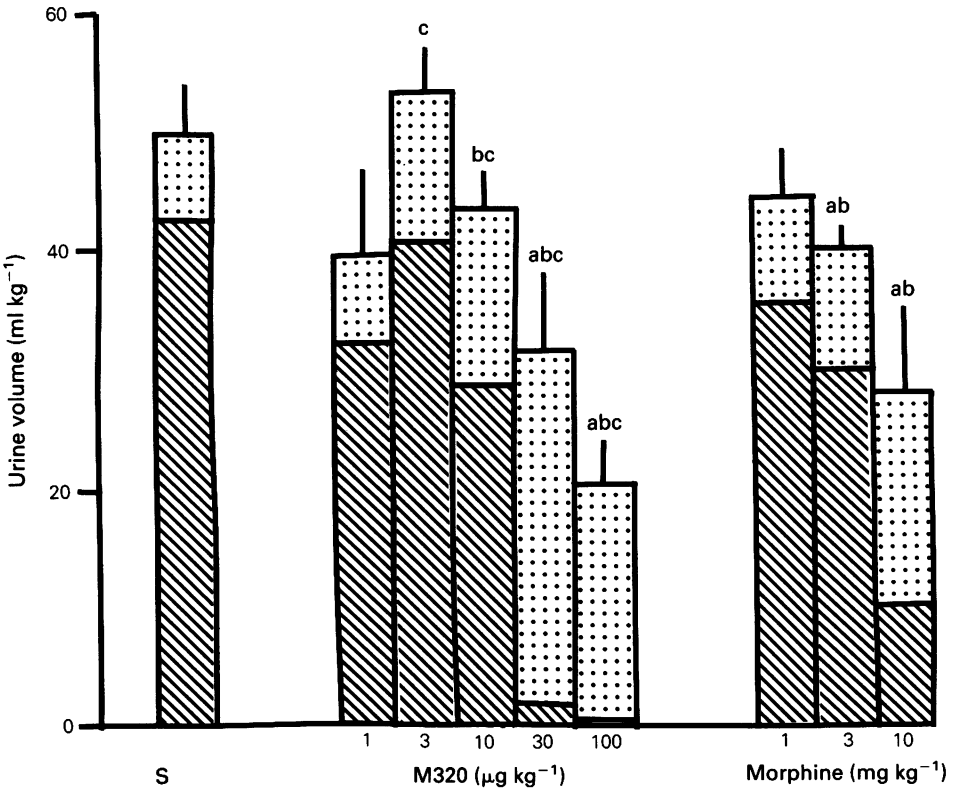

Figure 4 Urine production by normally hydrated male Brattleboro rats in the 4 h period following treatment with saline (S), M320 or morphine subcutaneously. The total volume of urine produced (entire column) is subdivided into urine voided (hatched area) and urine retained in the bladder (stippled column). Values are the means from 5–8 rats; a vertical line shows the s.e.mean of the total volume. [a] Represents significant difference from saline-treated animals for total volume of urine produced. [b] for urine voided and [c] for urine retained in the bladder ($P < 0.05$).

adsorption on to apparatus. Problems arising from adsorption were overcome by rigorous cleaning or disposal of apparatus between experiments. When using the rabbit vas deferens, cumulative dose-response curves were obtainable and produced comparable data to a single dose technique if one curve only was obtained from each preparation. Cumulative dose-response curves were therefore considered valid

Table 3 Plasma immunoreactive arginine vasopressin (ir-AVP) concentration of normally hydrated ($n = 6$) and 36 h water deprived rats ($n = 5$) 90 min after saline or M320 administration

State of hydration	*M320* (μg kg^{-1}, s.c.)	*Plasma ir-AVP* (pg ml^{-1})
Normally hydrated	0	2.38 ± 0.61
Deprived of water for 36 h	0	9.92 ± 1.57
	0.3	9.24 ± 2.44
	3	2.26 ± 0.58*
	30	Level in 4 out of 5 animals below limit of detection

*Values differ significantly from controls ($P < 0.05$).

and were used to produce the data shown in Figure 5.

Effect on the rabbit isolated vas deferens

Bremazocine and M320 inhibited the electrically evoked twitch of rabbit isolated prostatic vas deferens dose-dependently with IC_{50} values of 4.9 (2.6–8.5) and 1.7 (1.1–2.5) nM respectively (Figure 5). The depressant effect of M320 was extremely persistent and continued to be present for at least 1 h despite several washes. Neither morphine nor [Leu]enkephalin had any effect up to a concentration of 10 μM.

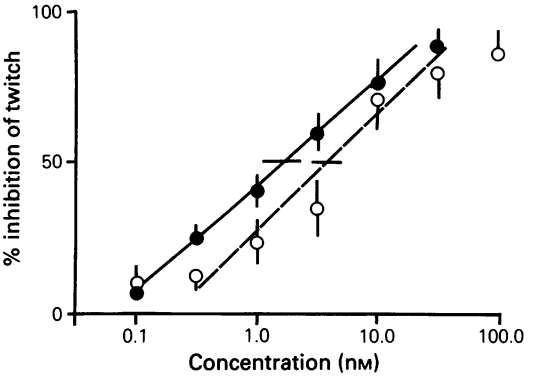

Figure 5 The effect of bremazocine (○) and M320 (●) on the rabbit isolated, electrically stimulated prostatic vas deferens. Points are the mean ($n = 4-7$); a vertical line shows the s.e.mean of the experimental points whilst the horizontal lines show 95% confidence limits of the calculated IC_{50} values.

Discussion

The present study confirmed the observation of Boura & Fitzgerald (1966) that M320 is a potent diuretic in rats after subcutaneous administration of 1.0–10 μg kg^{-1}. However, it was also found that the dose-response curve for the diuretic action of M320 was bell-shaped, doses greater than 30 μg kg^{-1} causing a much smaller effect than 10 μg kg^{-1}.

For technical reasons, *in vivo* experiments with M320 have been described using three observation periods. In the initial experiments urination was monitored from 0–2 h after treatment. Further studies utilized the period when the maximum effect of the drug was observed (30–150 min) whilst in the third series, data for urine voided in 4 h have been provided since the volume of urine retained in the bladder was measured at the completion of an experiment (4 h after treatment with M320). The volumes of urine voided are greater using the 4 h period of observation but analogous results were found using all protocols. Responses to 30 μg kg^{-1} M320 were the most variable and this is consistent with the steep descent of the bell-shaped dose-response curve between 10 and 100 μg kg^{-1}.

The bell-shaped curve for M320-induced urination could indicate that the drug has full κ-agonist activity with some μ-activity at higher doses. According to Leander (1983a, b), drug effects on urination can be used to discriminate between κ- and μ- opiate receptor activity, the former mediating diuretic and the latter antidiuretic effects. Additional evidence to support this hypothesis was sought using opiate receptor antagonists. In the whole animal the actions of M320 are slow to reach a maximal effect (90 min) but once established they cannot be easily reversed by antagonists (Boura & Fitzgerald, 1966). Naloxone has a short duration of action which led us to use naltrexone, a long lasting opiate antagonist which has some specificity for μ-receptors at lower doses and antagonizes both μ- and κ-receptors at higher doses (Takemori & Portoghese, 1984). It was possible to demonstrate clear dose-dependent antagonism of the diuretic activity of M320 by pretreatment with 1 and 10 mg kg^{-1} of naltrexone. This is consistent with the hypothesis that the diuresis caused by low doses of M320 is mediated by κ-receptors. The lack of diuresis following the high dose (30 μg kg^{-1}) of M320 can be interpreted as the resultant of opposing κ- and μ-receptor activation on urine output. The effect of 1.0 mg kg^{-1} naltrexone on this dose of M320 increased urine output suggesting that the more selective antagonism of the μ-receptor-mediated antidiuresis allowed κ-receptor-mediated diuresis to predominate. The highest dose of naltrexone used (10 mg kg^{-1}) can also be expected to antagonize κ-induced urine production, as was observed.

Further evidence that M320 has an agonist action at κ-receptors was obtained from *in vitro* studies. Oka *et al.*, (1980) showed the rabbit vas deferens to be a specific bioassay tissue for κ-agonists. The high potency and efficacy of M320 and the κ-agonist bremazocine (Romer *et al.*, 1980) on this tissue supports the contention that M320 is a potent agonist at κ-receptors. As expected the tissue was insensitive to the μ- and δ- agonists morphine and [Leu] enkephalin even in high concentrations.

The long duration of action (Boura & Fitzgerald, 1966), together with the fact that its effect persisted after washing suggest that M320 may form a durable bond with the κ-receptor.

The finding that low doses of M320 caused diuresis with only small increases in total sodium and potassium excretion indicated the possibility that the drug might lower plasma vasopressin levels. Evidence, both

indirect and direct, was obtained that suppression of endogenous vasopressin secretion was an essential prerequisite for its diuretic activity. The drug failed to induce diuresis in homozygous Brattleboro rats. Rats of this strain (DI/DI) are genetically deficient and are unable to synthesize and express AVP from their hypothalamus although expression of AVP is competent in the ovary (Clements *et al.*, 1985). Even when the high rate of urine production of the Brattleboro rat was reduced to normal by replacement therapy for 6 days, the drug still failed to cause diuresis. The data obtained thus suggested that a competent neurohypophysis was essential for M320-induced diuresis and furthermore that M320 caused no changes in either the metabolism of exogenous vasopressin or renal sensitivity to its antidiuretic effect.

Direct measurement of plasma ir-AVP showed that this was depressed in normally hydrated Long Evans rats treated with M320. When dehydration (36 h) was used to induce secretion of endogenous AVP, the elevated plasma levels of ir-AVP which resulted were reduced dose-dependently by M320. No bell-shaped dose-response curve was evident using doses comparable to those used in the diuresis experiments. Slizgi & Ludens (1982) reported analogous results for the effects of ethylketocyclazocine on plasma ir-AVP levels after using PEG 6000 (Sigma) to contract the plasma volume so stimulating vasopressin release.

Although the correlation between reduction of ir-AVP in the plasma, ability of the pituitary to secrete vasopressin and the diuretic activity of M320 in the normal rat is striking, Blackburn and co-workers (1985a, b) have postulated that the adrenal medulla plays a crucial role in the diuretic activity of tifluadom, an atypical benzodiazepine with agonist activity at κ-opiate receptors. Miller (1975) and Slizgi & Ludens (1982) provided evidence for the involvement of factors in addition to suppression of AVP in the overall diuretic activity of ethylketocyclazocine by comparing its effect with physiological suppression of AVP release (water load). Clarification of these aspects will require further investigation.

The antidiuretic activity of M320 is also of some interest. The diuresis caused by the normally non-diuretic dose ($30\,\mu g\,kg^{-1}$) of M320 in the presence of naltrexone together with complete suppression of plasma ir-AVP at that dose, indicated that increased urine formation was possibly proceeding although it was not being voided from the bladder. The pathological changes in the bladder described by Boura & Fitzgerald (1966) are consistent with this explanation. An attempt was made to analyse these components by using Brattleboro rats and, in addition, measure not only the volume of urine voided but also that retained in the bladder. The apparent reduction in urine volume when measured as voided volume after $10\,\mu g\,kg^{-1}$ M320 could be entirely accounted for by increased retention in the bladder. A reduction of the total amount of urine formed occurred only after high doses of M320. A similar study of normal Long Evans rats gave comparable results. The differences which did occur were probably due to the different basal levels of urine production caused by the differing levels of endogenous vasopressin secretion in the two strains.

The nature of the antidiuretic effects of μ-agonists is controversial. Early work by De Bodo (1944) and Duke *et al.*, (1951) indicated that, at least in the dog, the effect involved an increase in AVP release from the posterior pituitary.

Huidobro & Huidobro-Toro (1979), however, showed that the characteristics of morphine-induced antidiuresis in the rat (reduced urine volume together with reduced sodium and potassium concentrations) were inconsistent with the hypothesis that the antidiuresis was due to increased AVP secretion. They showed that administration of exogenous vasopressin also reduced the urine volume but this was accompanied by an increase in urinary sodium and potassium concentrations. In addition, Huidoboro-Toro (1980), demonstrated that both morphine and β-endorphin had antidiuretic effects in homozygous Brattleboro rats which were accompanied by decreased sodium and potassium excretion. Our own data indicate that the antidiuresis following high doses of M320 is not accompanied by any increase in plasma levels of ir-AVP. They were, in fact, lower than control values in untreated rats.

Wilson & Ngsee (1982) demonstrated that the antidiuretic effect of morphine in conscious normal Long Evans rats and homozygous Brattleboro rats was accompanied by a significant decrease in mean arterial pressure which may be a contributory element in its effects on urine output.

Recently Dray & Metsch (1984 a, b, c) have investigated the effect of a number of opioids on bladder function in ketamine anaesthetized rats. They concluded that opioids acting at μ- and perhaps also δ-receptors inhibit urinary bladder motility by a central mechanism, whereas the κ-receptor agonist U-50, 488 was inactive. Thus, the antidiuretic effect of μ-agonists at opioid receptors is not consistent with increased secretion of AVP from the posterior pituitary but may involve haemodynamic effects and central control of bladder and vesical sphincter function.

The present study investigated the physiological mechanisms and opiate receptor subtypes involved in the diuretic and antidiuretic effects of M320 in rats. The indications are that M320 may cause diuresis at doses of $1-10\,\mu g\,kg^{-1}$ s.c. by inhibiting posterior pituitary AVP release via activation of κ-opioid receptors. The antidiuretic effect of high doses of M320 appears to involve a number of factors associated with activation of central μ-opioid receptors.

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