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European Journal of Pharmacology 450 (2002) 153–162

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Modulation of formalin-induced behaviors and edema by local and systemic administration of dextromethorphan, memantine and ketamine

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Received 15 April 2002; received in revised form 8 July 2002; accepted 12 July 2002

Abstract

The present study examined the effects of local peripheral and systemic administration of three clinically used excitatory amino acid receptor antagonists (dextromethorphan, memantine, ketamine) on pain behaviors and edema produced by formalin (1.5% and 5%) in rats. Peripheral administration of dextromethorphan produced a locally mediated suppression of flinching behaviors induced by 1.5% and 5% formalin, but biting/licking behaviors were not affected. Memantine and ketamine had no effect on either of these behaviors. All three agents augmented edema produced by 1.5% and 5% formalin. When administered alone, dextromethorphan, memantine and ketamine produced an intrinsic paw swelling response, and this was blocked by the biogenic amine receptor antagonists mepyramine, phentolamine, methysergide and ketanserin. Following systemic administration, all three agents suppressed biting/licking behaviors, had no effect on flinching behaviors, and suppressed paw swelling induced by 5% formalin to varying degrees. These results provide evidence for a peripherally mediated antinociceptive action of dextromethorphan in the rat formalin test, but this may not necessarily be due to block of excitatory amino acid receptors as it is not observed with memantine or ketamine. All three agents produce a peripherally mediated paw swelling, which is likely due to blockade of biogenic amine reuptake. Systemic administration of all three agents produces antinociceptive and anti-inflammatory actions that may be due to block of excitatory amino acid receptors in the spinal cord.

Keywords: Dextromethorphan; Memantine; Ketamine; Formalin; Edema

1. Introduction

For some time, the spinal cord has been recognized as a target for excitatory amino acid receptor antagonists in producing analgesia in inflammatory and neuropathic pain states (Dickenson, 1994; Price et al., 1994). More recently, it has been appreciated that peripheral excitatory amino acid receptors can regulate inflammatory pain. Thus, the local peripheral administration of agonists for such receptors can produce hyperalgesia and allodynia (Jackson et al., 1995; Zhou et al., 1996; Lawland et al., 1997), while local administration of antagonists produces antihyperalgesic and analgesic actions (Jackson et al., 1995; Davidson et al., 1997; Lawland et al., 1997; Davidson and Carlton, 1998). Excitatory amino acid receptors are present on sensory axons (Coggeshall and Carlton, 1998) and sympathetic postganglionic efferents (Coggeshall and Carlton, 1999), and these are upregulated following inflammation (Carlton and Coggeshall, 1999; Coggeshall and Carlton, 1999). Such observations have led to the suggestion that peripheral excitatory amino acid receptors may represent a novel target for analgesic therapies by localized or topical administration of excitatory amino acid receptor antagonists (Carlton, 2001).

In the present study, we have examined the effects of peripheral administration of three clinically used *N*-methyl-D-aspartate (NMDA) receptor antagonists (dextromethorphan, memantine, ketamine) on pain behaviors produced by two different concentrations of formalin (1.5% and 5%). The formalin test is widely used as a model of acute inflammatory pain. Formalin injection activates peripheral sensory nerves (Puig and Sorkin, 1995; McCall et al., 1996) and produces pain behaviors that involve ongoing peripheral activity and peripheral and central sensitization (Tjølsen et al., 1992; Coderre et al., 1993b; Dallel et al., 1995). The formalin test generally uses concentrations ranging from 0.5% to 5%, and while pain behaviors are dose-related over this range (Coderre et al., 1993a; Abbott et al., 1995),

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PII: S0014-2999(02)02119-2

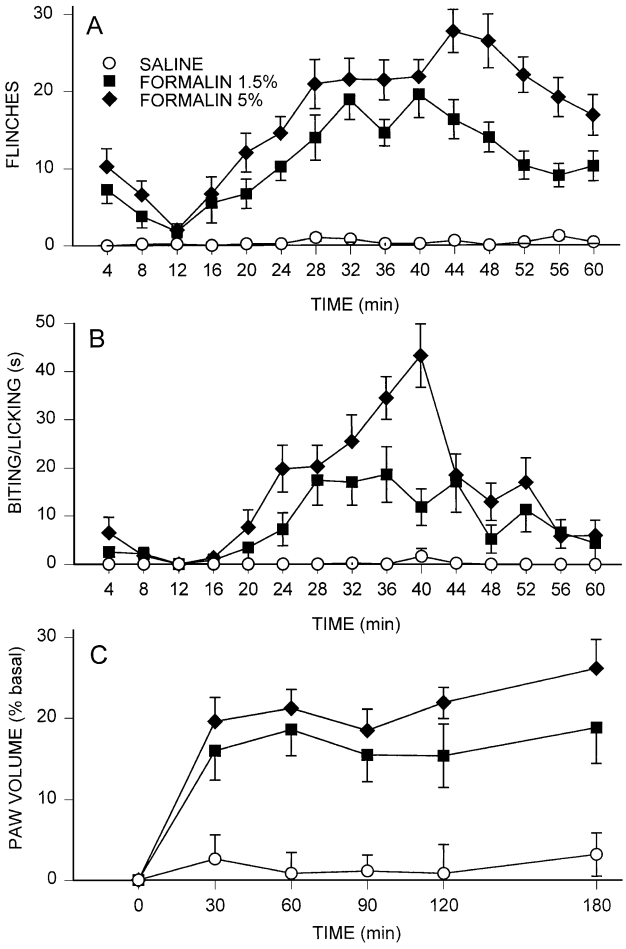

Fig. 1. Time course of (A) flinching behaviors, (B) biting/licking behaviors and (C) increase in paw volume produced by 1.5% and 5% formalin. Formalin was injected in 50 μl s.c. into the dorsal aspect of the hindpaw. Data for behaviors represent pooled control values from peripheral analgesia experiments (formalin $n=18$, saline $n=5$). Data for paw volume obtained in a separate experiment ($n=7$ per group). Values are mean ± S.E.M.

different mechanisms are involved at low and high concentrations. Thus, at low concentrations (<2%), there is a predominant activity of capsaicin-sensitive neurogenic components, while at a high concentration (5%), there is the additional involvement of more complex inflammatory elements (Damas and Liégois, 1999). Only the high concentration of formalin (5%) activates microglia in the spinal cord and produces a long-term hyperalgesia (Fu et al., 2000), and drugs that inhibit prostaglandin synthesis have a selective effect at 5% formalin (Yashpal and Coderre, 1998). It was demonstrated recently that 5% formalin, but not 2% formalin, releases glutamate from the rat spinal cord (Okuda et al., 2001). Five-percent formalin also releases glutamate locally in the rat hindpaw, but data for a lower concentration were not reported (Omote et al., 1998). If peripheral release of glutamate is also selective for a high concentration of formalin, then local peripheral administration of NMDA receptor antagonists could exert a selective action at high, but not low, formalin concentrations. On the other hand, dextro-

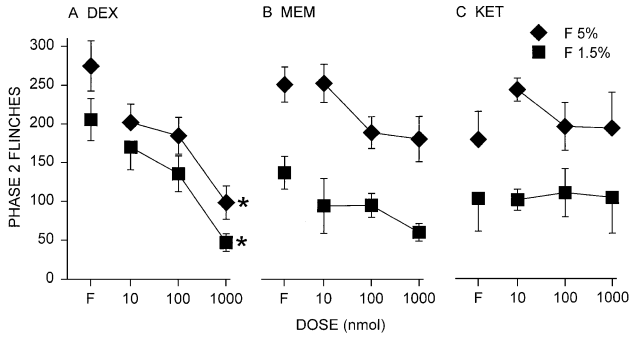

Fig. 2. Effects of local peripheral administration of (A) dextromethorphan (DEX), (B) memantine (MEM) and (C) ketamine (KET) on phase 2 (16–60 min) flinching behaviors produced by 1.5% and 5% formalin. Drugs were injected in 50 μl 10 min prior to the formalin. Values are mean ± S.E.M. ($n=6$ per group). * $P<0.05$ compared to formalin.

methorphan, memantine and ketamine all exert multiple pharmacological actions in addition to blocking NMDA receptors (see Discussion), and other mechanisms might have a selective expression at lower formalin concentrations where neurogenic mechanisms are predominantly involved and there is little concern for ceiling effects or behavioral asymptotes (cf. Abbott et al., 1995) obscuring drug actions. Dextromethorphan (Bern and Peck, 1992), memantine (Parsons et al., 1999) and ketamine (Reich and Silvay, 1989) are currently in clinical use, and these agents represent the first line of NMDA receptor antagonists that might be used for local peripheral application (e.g. as topical analgesics) to control pain. Each of these agents produces analgesia following systemic and spinal administration in various preclinical and clinical paradigms (Fisher et al., 2000), but their peripheral effects have received limited attention.

The local injection of formalin also produces an inflammatory response resulting in paw swelling (Wheeler-Aceto et al., 1990; Damas and Liégois, 1999). Spinal administration of excitatory amino acid receptor antagonists can regulate peripheral inflammatory responses (Sluka and Westlund, 1993), but there is little data on a peripheral contribution of

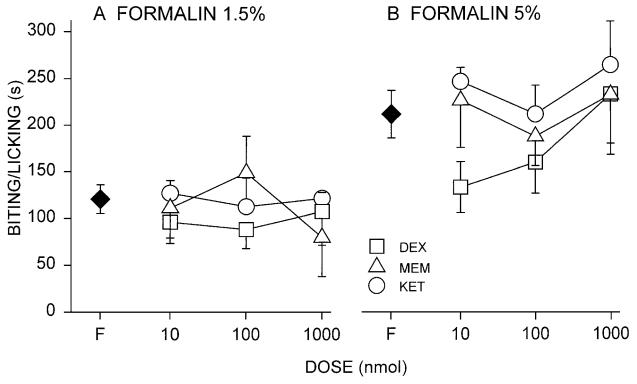

Fig. 3. Effects of local peripheral administration of dextromethorphan (DEX), memantine (MEM) and ketamine (KET) on phase 2 (16–60 min) biting/licking behaviors produced by (A) 1.5% and (B) 5% formalin. Values are mean ± S.E.M. ($n=6$ per group, but pooled controls for $n=18$).

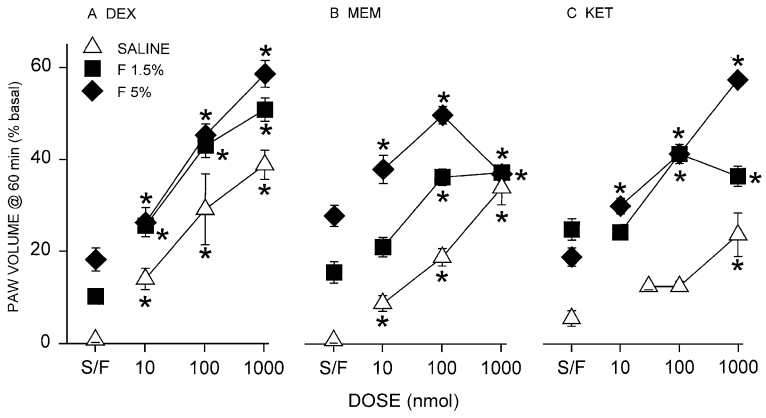

Fig. 4. Increase in paw volume at 60 min induced by 1.5% and 5% formalin in the absence and presence of (A) dextromethorphan (DEX), (B) memantine (MEM) and (C) ketamine (KET). Hollow symbols indicate intrinsic effect of drug at 60 min (see Fig. 6). Values are mean ± S.E.M. ($n = 6$ per group). * $P < 0.05$ compared to respective formalin (F) or saline (S) groups.

glutamate to this process. Given that glutamate can stimulate sensory afferent nerves (Du et al., 2001), it could potentially contribute to inflammation by recruiting a neurogenic mechanism. We therefore also determined the effects of dextromethorphan, memantine and ketamine on edema produced by formalin at 1.5% and 5% formalin. When an increase in edema was observed, intrinsic effects of these agents on paw volume, and potential mechanisms involved, were examined.

2. Materials and methods

Experiments were performed according to a protocol approved by the University Committee on Laboratory Animals in accordance with Canadian Council on Animal Care guidelines.

2.1. Animals

Male, Sprague–Dawley rats 150–200 g (Charles River, Quebec) were used in all experiments. Rats were housed under standard conditions at 22 ± 1 °C with a 12:12 h light/dark cycle.

2.2. Formalin test

Formalin, 1.5% or 5%, was injected subcutaneously in a volume of 50 μl into the dorsal aspect of the rat hindpaw. Rats were initially acclimated to the test chamber (Plexiglass container 28 × 28 × 28 cm) for 20 min prior to the injection, and returned to the chamber following injection. Both flinches (the number of paw elevations, rapid paw shakes or ripples of the haunch, with episodes of the latter two scoring as a single episode) and biting/licking time were recorded in 2-min intervals for 60 min following injection. Two rats were scored at a time in alternate 2-min bins. Behaviors were expressed as the total incidence during phase 1 (0–12 min) and phase 2 (16–60 min).

2.3. Paw volume

Paw volume (edema) was determined by volume displacement using a commercially available plethysmometer (Ugo Basile). The hindpaw was immersed into an electrolyte solution to the junction of the hairy and non-hairy skin, and volume displacement determined electronically in triplicate at each time interval. Pre-drug absolute paw volumes

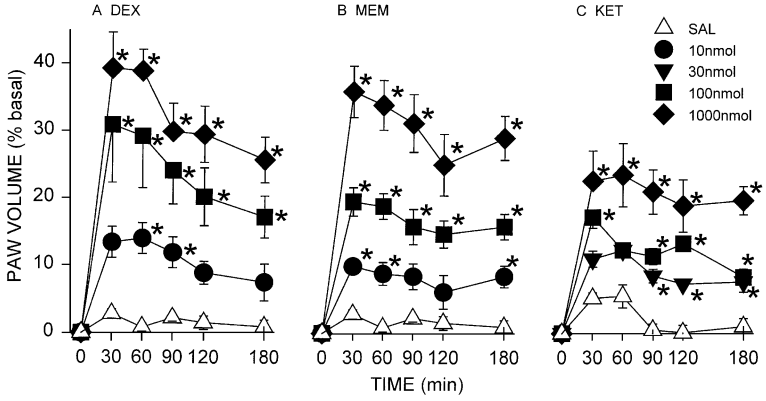

Fig. 5. Time course of increase in paw volume produced by (A) dextromethorphan (DEX), (B) memantine (MEM) and (C) ketamine (KET). Values are mean ± S.E.M. ($n = 6$ per group). * $P < 0.05$ compared to saline.

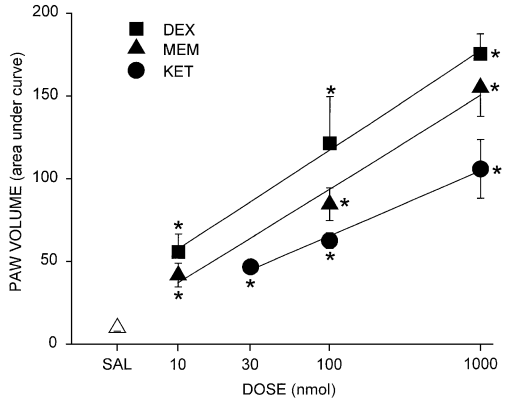

Fig. 6. Comparison of the increase in paw volume produced by dextromethorphan (DEX), memantine (MEM) and ketamine (KET). * $P<0.05$ compared to saline (SAL).

were determined prior to drug or formalin administration, and then again at the end of the 60-min behavioral scoring interval, and this was considered to adequately reflect the degree of swelling that occurred (cf. Fig. 1). For time course experiments, paw volumes were determined at 30, 60, 90, 120 and 180 min following injection. In the latter experiments, rats were used a second time after 3–5 days, with paw volume determinations in opposite paws. In all cases, paw swelling completely resolved within 24 h, and there were no residual effects.

2.4. Drug administration

For local peripheral administration, dextromethorphan hydrochloride, memantine hydrochloride and ketamine hydrochloride (Sigma) were administered in a volume of 50 μl saline s.c. into the dorsal surface of the hindpaw 10 min prior to the formalin. Formalin was injected in 50 μl s.c. into the same site. For paw volume time course experiments, amine antagonists (mepyramine maleate, phentolamine hydrochloride, methysergide maleate, ketanserin tartrate) (Sigma) were coadministered with drugs. For systemic administration, drugs were administered by intraperitoneal (i.p.) injection 20 min prior to the formalin hindpaw injection.

2.5. Expression of data and statistics

Time course data represents alternate 2-min scoring intervals, and as this was not corrected, it represents about half of the real value. Scores generated for flinching and biting/licking behaviors and paw volume were analyzed by ANOVA followed by the Student–Newman–Keul's test. Baseline paw volumes were 1.4–1.9 ml, and paw volumes are expressed as a percentage of the baseline value.

3. Results

3.1. Effects of formalin on pain behaviors and paw volume

The local injection of 1.5% and 5% formalin produces an increase in both flinching (Fig. 1A) and biting/licking behaviors (Fig. 1B). These behaviors are observed in two distinct phases (phase 1 = 0–12 min, phase 2 = 16–60 min) following injection, with the latter, but not the earlier, phase being dose related ($P<0.05$). Injection of these concentrations of formalin also produces a significant increase in paw volume, and this is expressed over a longer time interval (Fig. 1C). Both doses of formalin produced a similar degree of paw swelling.

3.2. Effects of local peripheral pretreatment with dextromethorphan, memantine and ketamine on formalin-evoked pain behaviors

Pretreatment with 10–1000 nmol dextromethorphan produced a suppression of phase 2 flinching behaviors produced by both 1.5% and 5% formalin (Fig. 2A). The effect of the 1000 nmol dose is due to a local action, as injection of this

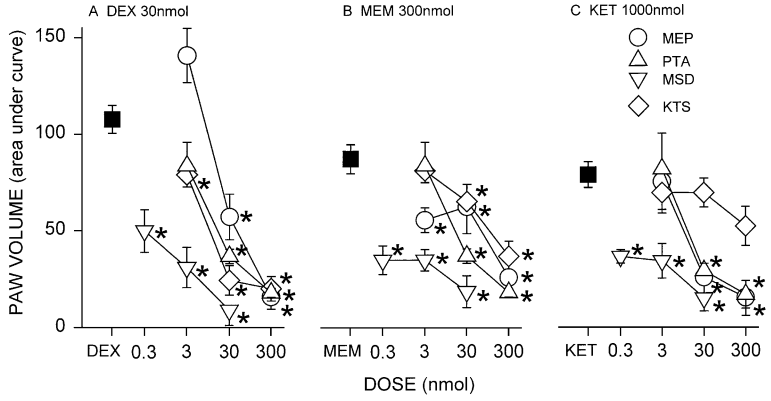

Fig. 7. Inhibition of the increase in paw volume produced by (A) dextromethorphan (DEX), (B) memantine (MEM) and (C) ketamine (KET) by the biogenic amine antagonists mepyramine (MEP; histamine H_1 receptor antagonist), phentolamine (PTA; α_1- and α_2-adrenoceptor antagonist), methysergide (MSD; 5-HT_1 and 5-HT_2 receptor antagonist) and ketanserin (KTS; 5-HT_2 receptor antagonist). Values are mean ± S.E.M. ($n=5–6$ per group, but pooled controls for $n=18$). * $P<0.05$ compared to respective control group.

dose into the contralateral paw had no effect on formalin-evoked behaviors (data not shown). Memantine had a minimal effect on flinching behaviors at the highest dose (Fig. 2B), while ketamine had no effect on flinching behaviors (Fig. 2C). All three drugs had no effect on phase 2 biting/licking behaviors (Fig. 3). None of the drugs had any significant effect on phase 1 behaviors, so these responses are not presented.

3.3. Effects of local peripheral administration of dextromethorphan, memantine and ketamine on paw volume

When paw volumes were determined at the end of the behavioral scoring interval, dextromethorphan, memantine and ketamine all produced a significant increase in the paw swelling induced by formalin 1.5% and 5% (Fig. 4). The effect of the highest dose of memantine (1000 nmol) exhibited a less than expected effect (Fig. 4B), suggesting a systemic action at the highest dose. When intrinsic effects of dextromethorphan, memantine and ketamine were examined in the absence of formalin, each agent produced a dose-related increase in edema (Figs. 4 and 5). The marked paw swelling that occurred with the combination of drug and formalin appeared to represent an additive effect between the drug and the formalin (Fig. 4). Dextromethorphan generally was the most active in producing edema (Figs. 5 and 6).

Dextromethorphan, memantine and ketamine all inhibit the uptake of biogenic amines (see Discussion), so the potential role of biogenic amines in paw swelling was examined. This was done by determining the effects of mepyramine (histamine H_1 receptor antagonist), phentolamine (α_1- and α_2-adrenoceptor antagonist), methysergide (5-HT_1 and 5-HT_2 receptor antagonist) and ketanserin (5-HT_2 receptor antagonist) on paw swelling. These antagonists all produced a dose-related inhibition of edema produced by dextromethorphan (Fig. 7A), memantine (Fig. 7B) and ketamine (except ketanserin) (Fig. 7C). A previous study demonstrated that these antagonists were without intrinsic effects on paw volume (Sawynok et al., 1999).

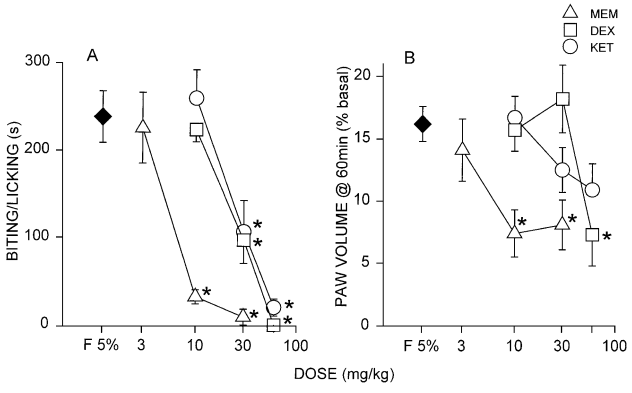

Fig. 8. Effects of systemic administration of memantine (MEM), dextromethorphan (DEX) and ketamine (KET) on (A) phase 2 biting/licking behaviors, and (B) paw swelling produced by 5% formalin (F). Values are mean ± S.E.M. ($n = 5–7$ per group, but $n = 10$ for formalin). * $P < 0.05$ compared to formalin group.

Table 1
Summary of motor effects observed with systemic administration of higher doses of dextromethorphan, memantine and ketamine

Dose	30 mg/kg	60 mg/kg
Memantine	sedation for 15–20 min, then hyperactivity and incoordination for 20–60 min	(not done)
Dextromethorphan	no effect	sedation for 40–50 min
Ketamine	motor incoordination for 15 min	motor incoordination for 30 min

3.4. Effects of systemic administration of dextromethorphan, memantine and ketamine on formalin-induced pain behaviors and paw volume

Effects of systemic administration of these agents were determined as a further verification of the local nature of drug actions when injected into the hindpaw. We were also interested in determining whether the effect on paw volume at the highest dose of memantine was due to a systemic action. Systemic administration of dextromethorphan, memantine and ketamine generally had no effect on flinching behaviors (data not shown). However, all three drugs uniformly suppressed phase 2 biting/licking behaviors, with memantine being the most potent (Fig. 8A). Phase 1 behaviors were unaffected by systemic drug treatments (data not shown). Memantine and dextromethorphan, but not ketamine, suppressed the edema produced by 5% formalin (Fig. 8B).

For each of the antagonists, significant motor effects were observed following systemic administration of higher doses (Table 1). No motor effects were observed following the 10 mg/kg doses injected systemically, or following local injections into the hindpaw.

4. Discussion

The present study examined the effects of local peripheral administration of dextromethorphan, memantine and ketamine on behavioral and inflammatory responses produced by two different concentrations of formalin (1.5% and 5%). It demonstrates (a) that dextromethorphan, but not memantine or ketamine, produces a peripherally mediated antinociception against flinching (but not biting/licking) behaviors; (b) that dextromethorphan, memantine and ketamine all enhance the paw swelling produced by formalin and induce a significant intrinsic paw swelling response, which can be blocked by biogenic amine receptor antagonists; and (c) that systemic administration of dextromethorphan, memantine and ketamine produces antinociception against 5% formalin-induced biting/licking (but not flinching) behaviors, and inhibits the increase in paw volume produced by formalin.

4.1. Local effects of dextromethorphan, memantine and ketamine on pain behaviors produced by formalin

There is now considerable evidence that peripheral ionotropic excitatory amino acid receptors are present on sensory afferent nerve terminals, and that activation of such receptors enhances activity in sensory afferent neurons and facilitates pain signaling (Carlton, 2001). The administration of 5% formalin into the rat hindpaw results in release of glutamate both peripherally (Omote et al., 1998) and spinally (Okuda et al., 2001). A lower concentration of formalin (2%) does not induce spinal release of glutamate, but no data were reported for the periphery. Dextromethorphan, memantine and ketamine each inhibit NMDA receptor activity by actions within the ion channel (Tortella et al., 1989; Parsons et al., 1999; Hirota and Lambert, 1996; Fisher et al., 2000). In view of these observations, we expected that these agents would produce a local antinociception at 5%, but perhaps not at 1.5%, formalin, since for an antagonist to produce a pharmacological effect, there must be significant agonist presence and activity. However, only dextromethorphan produced a local peripheral antinociception, and there was no difference in the degree of response at the two formalin concentrations. Given that all three agents block NMDA receptors (cf. Fisher et al., 2000), yet only one of them produces a peripherally mediated antinociception in the formalin test, it is possible that the activity of dextromethorphan depends upon pharmacological actions other than a block of NMDA receptors (see below).

The lack of effect with memantine and ketamine was surprising, as these agents previously had been reported to inhibit formalin-evoked behaviors (lifting/licking) following local peripheral administration (Davidson and Carlton, 1998). The two studies are similar with respect to doses used, formalin concentration and subjects, but differ with respect to injection site (dorsal versus plantar injection). A recent systematic comparison of behavioral and neurochemical effects following injection of 5% formalin into the plantar and dorsal hindpaw indicates that most behavioral outcomes are similar following the two methods of delivery (Okuda et al., 2001). While 5% formalin injected at both sites increased spinal cord glutamate release, the increase following plantar injection was greater than that following dorsal injection (Okuda et al., 2001). It is not clear if a differential effect in *peripheral* glutamate release following plantar versus dorsal injection occurs, and whether this could account for the behavioral differences between our study and that of Davidson and Carlton (1998).

An additional factor to consider in accounting for the lack of effect of NMDA receptor antagonists in the present study is the magnitude of the contribution of glutamate to afferent signaling following formalin injection. Thus, while glutamate can cause nociceptor excitation, the magnitude of this effect (1 and 0.36 impulses/s in Aδ and C fibres, respectively; Du et al., 2001) is lower than that produced by 5% formalin (up to 11 impulses/s; Puig and Sorkin, 1995). The lesser strength of activation may account for why local injections of glutamate do not lead to spontaneous pain behaviors (Carlton et al., 1998) as does formalin. The injection of 5% formalin results in a local inflammatory response involving multiple tissue mediators (Tjølsen et al., 1992), and it may be that the contribution of glutamate to afferent signaling is obscured amongst these multiple influences. Peripheral antinociception by NMDA receptor antagonists may be most clearly expressed in conditions where the contribution of glutamate to pain signaling is more prominently expressed. For example, inflammation with complete Freund's adjuvant causes an upregulation of ionotropic glutamate receptors on sensory afferents (Carlton and Coggeshall, 1999) and on sympathetic postganglionic nerves (Coggeshall and Carlton, 1999), and more chronic inflammatory conditions may be required to observe this influence.

In the present study, dextromethorphan was the only agent to produce a peripheral antinociception against flinching behaviors. Dextromethorphan can block NMDA receptors in a noncompetitive manner by actions within the ion channel (Tortella et al., 1989; Fisher et al., 2000). It can also block voltage-gated Ca^{2+} channels (Carpenter et al., 1988), and this action might contribute to antinociception at peripheral sites (cf. White and Cousins, 1998). It is interesting to note that dextrorphan, which is the demethylated metabolite of dextromethorphan, produced the most potent peripheral antinociception in the study by Davidson and Carlton (1998). While metabolism of dextromethorphan to dextrorphan may contribute to pharmacological actions following systemic administration (Wu et al., 1995), this is less likely following local peripheral administration into the hindpaw. The unique or enhanced activity of dextromethorphan and dextrorphan with regards to peripheral antinociception may reflect properties common to these two agents, but the nature of these is not entirely clear. Thus, both memantine (Parsons et al., 1999) and ketamine (Hirota and Lambert, 1996; Meller, 1996) also block Ca^{2+} channels, Na^{+} channels and cholinergic receptors, in addition to blocking NMDA receptors, yet neither of these agents exhibits prominent effects on formalin-evoked behaviors following local administration. It is important to appreciate that while actions on ion channels and other receptors may require concentrations that are higher than those required to block NMDA receptors, and may contribute only minimally to actions following systemic administration (Eide et al., 1998), local tissue concentrations following direct injection into the rat hindpaw are higher (1000 nmol is 50 μl of a 20 mM solution), and likely are in the range of doses that do recruit these additional mechanisms.

4.2. Effects of dextromethorphan, memantine and ketamine on paw volume

Dextromethorphan, memantine and ketamine all produced a dose-dependent augmentation of the paw swelling by formalin. While mechanisms involved in paw swelling at 1.5% and 5% formalin may be different (see Introduction),

the increase in paw volume occurs to a similar extent at both concentrations. In the absence of formalin, dextromethorphan, memantine and ketamine produce an intrinsic edema, with dextromethorphan being the most active in provoking this response. This intrinsic effect is of a magnitude that can fully account for the increase in paw volume in the presence of formalin. Ketamine has been known for some time to inhibit the uptake of biogenic amines (Smith et al., 1975; Taube et al., 1975; Azzaro and Smith, 1977), and this has been confirmed more recently using cloned monoamine transporters (Hara et al., 1998; Nishimura et al., 1998). Dextromethorphan inhibits the uptake of 5-HT (Ahtee, 1975; Meoni et al., 1997) and noradrenaline (Pubill et al., 1998a,b), and memantine inhibits a monoamine transporter that can transport noradrenaline, histamine, dopamine and 5-HT (Busch et al., 1998). As the local injection of histamine and 5-HT produces edema (Maling et al., 1974), we examined the potential involvement of biogenic amines in the paw swelling response. Methysergide (5-HT_1 and 5-HT_2 receptor antagonist), ketanserin (5-HT_2 receptor antagonist), phentolamine (α_1- and α_2-adrenoceptor antagonist) and mepyramine (histamine H_1 receptor antagonist) all inhibited paw swelling produced by these agents, and these observations implicate 5-HT, noradrenaline and histamine in paw swelling. The 5-HT involvement could reflect inhibition of 5-HT uptake following release from platelets; noradrenaline, the inhibition of uptake following release from sympathetic postganglionic neurons; and histamine, the inhibition of uptake following release from mast cells.

NMDA can exhibit functional interactions with monoamine mediated responses, and this represents another potential mechanism by which biogenic amines could be involved in paw swelling. Thus, both dextromethorphan and ketamine enhance 5-HT_2 receptor-mediated behaviors in mice (Kim et al., 1998), and as these are still prominent following monoamine depletion (Kim et al., 2000), this likely reflects functional links between central NMDA and 5-HT receptors. In the present experiments, the involvement of biogenic amines more likely reflects interactions with biogenic amine transporters as noted above, rather than such functional links. Thus, while NMDA receptors activate sensory afferents by peripheral actions (Du et al., 2001), and potentially could contribute to a neurogenic inflammation, the local administration of glutamate into the knee joint has no effect on knee joint diameter (Lawland et al., 1997). With no ready explanation as to how blocking peripheral excitatory amino acid receptors would generate paw swelling and edema, it is difficult to formulate a functional link between peripheral NMDA and biogenic amine receptors to produce this response.

4.3. Systemic administration of dextromethorphan, memantine and ketamine

When administered systemically, all three agents produced a dose-related suppression of biting/licking behaviors, but generally did not affect flinching. The lack of alteration of flinching by dextromethorphan 10 mg/kg further supports the local nature of the response observed following peripheral injection (1000 nmol is approximately 2 mg/kg). All three agents produced significant motor effects at the highest doses, but the suppression of biting/licking behaviors occurred at doses lower than those producing motor effects. In previous studies, systemic dextromethorphan (Elliott et al., 1995), memantine (Eisenberg et al., 1993) and ketamine (Shimoyama et al., 1999) all inhibited various aspects of nociceptive behaviors produced by 5% formalin in mice and rats. Each of those studies also noted motor effects, with suppression of behaviors occurring at doses lower than those producing motor effects. The nature of the motor effects produced by dextromethorphan, memantine and ketamine was somewhat different (Table 1), and this is consistent with a previous report that examined motor effects of multiple types of excitatory amino acid receptor antagonist (Danysz et al., 1994).

In the present study, differential effects on flinching and biting/licking behaviors were obtained following both systemic and local peripheral administration, and this suggests these behaviors reflect different mechanisms and/or circuitry. The flinch response may be a spinal reflex, as it can be elicited following chronic spinalization (Coderre et al., 1994; but see Wheeler-Aceto and Cowan, 1991), while biting/licking may represent a more coordinated response involving supraspinal integration (Wheeler-Aceto and Cowan, 1991; Abbott et al., 1995). Curiously, the implantation of chronic cannulas can completely eliminate the expression of biting/licking behaviors in response to formalin, while having little influence on the expression of flinching responses, and this may reflect spinal elements of inflammation (Sawynok and Reid, in press). This further indicates that the two behaviors reflect different mechanisms.

In view of the above differences, it is curious to note that in rats, the *systemic* administration of dextromethorphan, memantine and ketamine suppresses biting/licking behaviors (but not flinching behaviors) (this study) while *spinal* administration of these same agents suppresses flinching behaviors (Chaplan et al., 1997). Memantine was more potent and more effective than dextromethorphan and ketamine in both studies. It has been argued that, following systemic administration, the analgesic properties of NMDA receptor antagonists reflect actions on mechanisms within the spinal cord involving central sensitization (Chaplan et al., 1997). However, the lack of parallel in the profile of these agents by systemic and spinal routes suggests that additional mechanisms, such as supraspinal actions, as well as interactions between spinal and supraspinal sites, also may be involved. Supraspinal actions could involve inhibition of NMDA receptors at, for example, thalamic sites (Kolhekar et al., 1997), or activation of descending pain inhibitory mechanisms involving biogenic amines (Kawamata et al., 2000). It is unlikely that the higher tissue levels required for peripheral actions to be expressed are attained following systemic administration, and pharmaco-

logical actions most likely reflect higher affinity mechanisms for each of these agents. A final contributor to systemic actions could be active metabolites. Thus, both ketamine (norketamine, Shimoyama et al., 1999) and dextromethorphan (dextrorphan, Wu et al., 1995) produce active metabolites, and these may be of particular significance when drugs are given by intraperitoneal injections.

All three agents also suppressed paw swelling induced by formalin when given systemically. Memantine was the most potent, dextromethorphan decreased paw swelling only at the highest dose, and ketamine had a minimal effect. Interestingly, when administered peripherally, memantine exhibited a bell-shaped curve, and this had suggested a systemic effect by modest doses of this agent. However, the peripheral 1000 nmol dose is 2 mg/kg, but systemic effects with memantine were not observed until 10 mg/kg following intraperitoneal injection. It is not clear whether this indicates there is also a local anti-inflammatory effect of memantine, or if methodological or kinetic factors are involved. With respect to mechanism, it is likely that the inhibition of the formalin-induced paw swelling produced by memantine and dextromethorphan reflects a spinal anti-inflammatory action of these agents involving block of spinal NMDA receptors (Bong et al., 1996; but see Sluka and Westlund, 1993).

4.4. Potential clinical relevance of observations

In view of the involvement of peripheral glutamate receptors in peripheral pain signaling, drugs that block glutamate receptors may represent a potential therapeutic target for development as peripherally acting analgesics (Carlton, 2001). Consistent with this suggestion are reports that peripheral administration of ketamine can reduce hyperalgesia (Warnke et al., 1997) or produce analgesia (Pedersen et al., 1998) in experimental burn pain paradigms in humans. There are also some case reports on the efficacy of topical ketamine in sympathetically maintained pain (Crowley et al., 1998) and in hospice patients (Wood, 2000). However, other studies have not observed peripheral analgesia with ketamine using the intradermal capsaicin model in humans (Koppert et al., 1999; Gottrup et al., 2000).

An important issue that arises from studies such as this is the utility of the formalin test as a potential model for clinical situations. The formalin test is an acute inflammatory model that involves both peripheral and central sensitization (Tjølsen et al., 1992; Coderre et al., 1993a; Dallel et al., 1995), and was originally developed as a model of persistent pain that was of greater relevance to clinical pain than phasic models (Dubuisson and Dennis, 1977). As inflammation can upregulate glutamate receptors on primary afferent neurons (Carlton and Coggeshall, 1999) and sympathetic postganglionic neurons (Coggeshall and Carlton, 1999), a more chronic inflammatory model, or a model involving a clear sympathetic component (e.g. neuropathic pain), may be required to reflect a clear peripheral analgesia by NMDA receptor antagonists. On the other hand, experimental burn pain in humans is also a model of acute inflammation, and this model reveals some antihyperalgesic (Warnke et al., 1997) and analgesic (Pedersen et al., 1998) properties of locally administered ketamine, although several pain parameters also were unaffected (Pedersen et al., 1998). No local peripheral analgesia was observed with ketamine using the intradermal capsaicin model, which is a model of peripheral and central sensitization (Koppert et al., 1999; Gottrup et al., 2000). In order to better appreciate the potential for this class of agents to represent a novel approach to the control of pain, it will be necessary to determine the analgesic profile of excitatory amino acid receptor antagonists in more chronic inflammatory pain models and neuropathic pain models in preclinical studies, as well as in further models of clinical pain.

A final implication of the present observations relates to potential adverse effects. Thus, local administration of dextromethorphan, memantine and ketamine all produces an intrinsic paw swelling, or edema, which likely results from plasma extravasation and may represent a pro-inflammatory potential for these agents. These observations suggest that local adverse effects following local peripheral administration of these agents should be monitored. There are two issues to note regarding these observations. (1) These responses could be species specific. Thus, biogenic amines including 5-HT are involved in the paw swelling produced by dextromethorphan, memantine and ketamine in rats. Rodent mast cells (rats, mice) contain both histamine and 5-HT, but human mast cells contain only histamine (Schwartz, 1994), so this effect might be more pronounced in rodents than in humans. It has, however, been noted that s.c. infusion of ketamine in humans can produce itching and painful indurations at the site of injection (Eide et al., 1995), and the prominence of the itch sensation suggests local accumulations of histamine in the tissue. (2) This response could depend on the route of administration. Thus, s.c. injection of saline induces a local release of histamine in rats (Guo et al., 1997), and administration by this route, involving mechanical perturbations to the tissue, may be required to see swelling. It remains to be seen whether this occurs following other local delivery methods (e.g. topically). The development of topical formulations of analgesic drugs has the important potential to produce fewer adverse systemic effects, but the possibility of producing local tissue and cutaneous adverse reactions must be considered.

Acknowledgements

This work was supported by grants from the Canadian Institutes of Health Research and EpiCept Corporation.

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