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Pain

Ketamine and norketamine attenuate oxycodone tolerance markedly less than that of morphine: from behaviour to drug availability

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Abstract

Background: Ketamine attenuates morphine tolerance by antagonising *N*-methyl-D-aspartate receptors. However, a pharmacokinetic interaction between morphine and ketamine has also been suggested. The interaction between oxycodone and ketamine is unclear. We studied the effects of ketamine and norketamine on the attenuation of morphine and oxycodone tolerance focusing on both the pharmacodynamic and pharmacokinetic interactions.

Methods: Morphine 9.6 mg day^{-1} or oxycodone 3.6 mg day^{-1} was delivered to Sprague–Dawley rats by subcutaneous pumps. Once tolerance had developed, the rats received subcutaneous injections of ketamine or norketamine. Tail-flick, hot-plate, and rotarod tests were performed. Drug concentrations were measured with high-performance liquid chromatography–tandem mass spectrometry.

Results: Anti-nociceptive tolerance to morphine and oxycodone developed similarly by Day 6. Acute ketamine 10 mg kg^{-1} and norketamine 30 mg kg^{-1} attenuated morphine tolerance for 120 and 150 min, respectively, whereas in oxycodone-tolerant rats the effect lasted only 60 min. Both ketamine and norketamine increased the brain and serum concentrations of morphine, and inhibited its metabolism to morphine-3-glucuronide, whereas oxycodone concentrations were not changed. Morphine, but not oxycodone, pretreatment increased the brain and serum concentrations of ketamine and norketamine. Ketamine, but not norketamine, significantly impaired the motor coordination.

Conclusions: Ketamine and norketamine attenuated morphine tolerance more effectively than oxycodone tolerance. Ketamine and norketamine increased morphine, but not oxycodone brain concentrations, which may partly explain this difference. Norketamine is effective in attenuating morphine tolerance with minor effects on motor coordination. These results warrant pharmacokinetic studies in patients who are co-treated with ketamine and opioids.

Keywords: anaesthetics; dissociative; analgesics; opioid; ketamine; morphine; oxycodone; pain; tolerance

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Editor's key points

- The effect of ketamine and its metabolite, norketamine, on oxycodone tolerance is unclear, although the effects on morphine tolerance are better characterised.
- Rats tolerant to morphine or oxycodone anti-nociception were given acute doses of ketamine or norketamine.
- Ketamine and norketamine reduced morphine and, to a lesser extent, oxycodone tolerance, associated with increased brain morphine, but not oxycodone concentrations.
- Ketamine, but not norketamine, affected motor coordination.
- Pharmacokinetic studies in patients co-treated with ketamine and opioids are required.

The dissociative anaesthetic ketamine and opioids are frequently co-administered,[1] and ketamine inhibits morphine tolerance[2–4] mainly by *N*-methyl-D-aspartate (NMDA) receptor antagonism. Recently, we reported that, in morphine-tolerant rats, acutely administered ketamine markedly increased both morphine and ketamine concentrations.[5] Oxycodone is increasingly used as an analgesic in acute and cancer pain. However, little is known about its co-administration with ketamine, and nothing about possible pharmacokinetic interactions.

Norketamine, the primary metabolite of ketamine,[1] is a three to five times weaker NMDA receptor antagonist than ketamine.[6–9] In pre-clinical models, norketamine has antinociceptive properties,[9–11] but the effects of norketamine on existing opioid tolerance have not been studied. Estimations of the contribution of norketamine to clinical ketamine analgesia are controversial.[12–14]

Both treatment response and adverse effects vary significantly when ketamine is combined with opioids as a strategy to manage cancer pain, probably because of the heterogeneity of patients, different routes of administration, and the opioid used.[15,16] Evidence supports the perioperative use of low-dose ketamine with morphine,[17] whereas there is a lack of evidence to support its use in cancer pain.[15,16] We hypothesized that the efficacy of ketamine on opioid tolerance varies, depending on the opioid used because of pharmacokinetic factors and that, when treating opioid tolerance, norketamine may have advantages over ketamine in terms of efficacy and adverse effects. We studied the pharmacodynamic and pharmacokinetic effects of ketamine or norketamine co-administration in morphine- and oxycodone-tolerant rats.

Methods

Animals

All procedures were in accordance with the guidelines of The International Association for the Study of Pain[18] and are reported in accordance with the Animal Research: Reporting of In Vivo Experiments guidelines, with approval of the provincial government of Southern Finland (ESAVI/7929/04.10.07/2014) and complying with EU2010/63 legislation. Referring to Annex IV/Table 3, we followed the recommendation that decapitation is an acceptable method if other methods cannot be considered, as in the current case. Male Sprague–Dawley rats (Harlan, Horst, The Netherlands; 200–250 g, n=6–12 per group in behavioural studies and n=4–7 in concentration measurements; total n=165) were housed in groups of four in specific pathogen-free cages in light- and temperature-controlled rooms (lights on at 07:00 h, off at 19:00 h; temperature 22 (2)°C). Normal water and standard pellets were available *ad libitum*. After behavioural experiments, the rats were culled by guillotine decapitation, and tissue samples were collected.

Drugs

Oxycodone hydrochloride was purchased from Sigma-Aldrich (St Louis, MO, USA), morphine hydrochloride and racemic ketamine hydrochloride (Ketaminol vet.; Boxmeer, Netherlands) from University Pharmacy (Helsinki, Finland), and racemic norketamine hydrochloride from Tocris (Bristol, UK). Morphine, norketamine, and oxycodone were dissolved, and ketamine was diluted in physiological saline and administered subcutaneously (s.c.) in a volume of 2 ml kg^{-1}. All drug concentrations were expressed as free base amounts.

Opioid-tolerance scheme

Morphine and oxycodone tolerance was induced during 6 days with continuous opioid administration via osmotic minipumps (Alzet 2ML1; DURECT, Cupertino, CA, USA). The pumps were filled with oxycodone 15 mg ml^{-1} to deliver oxycodone 3.6 mg day^{-1}, or morphine 40 mg ml^{-1} to deliver morphine 9.6 mg day^{-1}. The pumps were not pre-primed and the treatment lasted for 7 days. The drugs were diluted in sterile water. Sterile water pumps were used as a control. The pumps were s.c. implanted between the scapulae under brief isoflurane 3.0% anaesthesia. The adequacy of anaesthesia was ascertained by the lack of withdrawal response to tail pinch. The health of the rats was monitored after operation, and no objective signs of pain or discomfort were observed.

Behavioural tests

All behavioural tests were conducted during the morning. After habituation for 3 days, nociception was assessed with tail-flick and hot-plate tests as described previously.[5] The baseline latencies for each day were measured before drug administrations. Rotarod tests were performed with Ugo Basile (Gemonio, Italy) 47700 Rat RotaRod apparatus, diameter 70 mm, with speed accelerating from 10 to 40 rpm during the course of 60 s. The rat was placed on the rotating rod, and the time it stayed on it was measured. The cut-off time was 60 s.

At each time point, the tests were performed in the same order. The tail-flick test was performed first, followed by the hot-plate test. The rotarod test was performed last. The animals were randomly assigned to treatment groups. The person performing the behavioural tests was blinded to the randomized treatments, which were given by another investigator.

Drug-concentration measurements

The rats were decapitated and whole blood was collected. The coagulated blood samples were centrifuged at 2000 *g* for 10 min at +4°C. Whole brain samples were snap frozen in liquid nitrogen and stored at −80°C. The collected serum was stored at −80°C. The concentrations of the opioids, ketamine, and their main metabolites were quantified by high-performance liquid chromatography–tandem mass spectrometry (see text, Supplementary Digital Content 1, for a detailed description).

Tolerance experiment

The outline of the tolerance experiment is shown in Supplementary Figure 1. On Day 0, rats were implanted with minipumps delivering morphine 9.6 mg day^{-1}, oxycodone 3.6 mg day^{-1}, or vehicle. The doses were chosen to induce similar acute anti-nociception and a similar development of anti-nociceptive tolerance. Tolerance was evaluated on Days 1, 3, and 6. On Day 6, after baseline measurements, the rats received ketamine 10 mg kg^{-1} s.c., norketamine 30 mg kg^{-1} s.c., or vehicle. Norketamine 10 mg kg^{-1} was also used in combination with morphine. Behaviour was monitored up to 150 min. The rotarod test was also performed 5, 10, and 15 min after drug administration.

Tissue samples for concentration measurements were collected on Day 7. The rats were given the same drugs as on Day 6, and samples were collected at 30 and 90 min after drug administration. Because the rats were culled at the time point of sample collection, the treatment groups had been allocated previously into two subgroups (30 and 90 min samples).

Acute experiment

The effects of ketamine or norketamine on oxycodone-induced anti-nociception in acute co-administration were studied. Rats received oxycodone 0.7 mg kg^{-1} s.c. at time point −15 min and s.c. racemic ketamine, norketamine, or vehicle at 0 min. To assess the potential pharmacokinetic interactions, after 1 week's washout, the same drugs were administered and tissue samples were acquired at 30 min.

Statistical analysis

One serum sample was excluded because of a handling error. The results of the nociceptive tests are expressed as percentage of the maximum possible effect (MPE%), calculated as MPE %=[(post-drug latency − baseline latency)/(cut-off time − baseline latency)] × 100%. The data are presented as mean (standard deviation). For the concentration data, individual data points are shown. The behavioural data were tested for statistically significant differences by two-way analysis of variance (ANOVA) followed by the Holm–Sidak *post hoc* analysis. For the concentration data, an unpaired two-tailed *t*-test or one-way ANOVA followed by the Holm–Sidak *post hoc* analysis was performed. The difference was considered significant at $P<0.05$ in both the ANOVA and the *post hoc* test. The data were analysed using GraphPad Prism, version 6.0h for Mac OS X (GraphPad Software, La Jolla, CA, USA).

Results

Development of tolerance

In the tail-flick (Fig. 1A and B) and hot-plate (Fig. 1C and D) tests, similar anti-nociception was seen on Day 1. Tolerance developed similarly in both the morphine- and oxycodone-treated groups. Both opioids caused a small decrease in rotarod performance on Days 1 and 3 (Fig. 1E). The effect of oxycodone remained constant until Day 6 (Fig. 1F).

Norketamine restores morphine antinociception without impairing rotarod performance

Norketamine 10 mg kg^{-1} s.c. administered to vehicle-treated rats had no effects on nociception or rotarod performance (Fig. 2A–C). In the hot-plate test, ketamine 10 mg kg^{-1} and norketamine 30 mg kg^{-1} caused similar anti-nociception at 30–60 min (Fig. 2B). Ketamine 10 mg kg^{-1} impaired rotarod performance at 5–10 min compared with norketamine 30 mg kg^{-1} (Fig. 2C). In morphine-tolerant rats, ketamine 10 mg kg^{-1} restored morphine anti-nociception at 30 min, and the effect lasted until 120 min in both the tail-flick (Fig. 2D) and hot-plate tests (Fig. 2E). Ketamine impaired rotarod performance at 5–15 min (Fig. 2F). Norketamine 30 mg kg^{-1} reversed morphine tolerance to the end of the measurement period (Fig. 2D and E) without effects on rotarod performance (Fig. 2F).

Weak attenuation of oxycodone tolerance by ketamine and norketamine

Norketamine 30 mg kg^{-1} and ketamine 10 mg kg^{-1} administered to vehicle-pretreated rats had no anti-nociceptive effects in the tail flick (Fig. 3A), but they had small effects in the hot-plate test (Fig. 3B). In the rotarod test (Fig. 3C), norketamine 30 mg kg^{-1} disturbed the motor coordination at 5–10 min. The effect of ketamine was more pronounced than that of norketamine and lasted up to 15 min. In oxycodone-tolerant rats, ketamine caused significant anti-nociception only at 60 min (Fig. 3D), whereas the effect of norketamine was significant at 30–60 min in the tail-flick test. In the hot-plate test (Fig. 3E), both ketamine and norketamine significantly attenuated tolerance at 30 and 60 min, with ketamine being more potent at the 30 min measurement point. Ketamine and norketamine disturbed motor coordination at 5–15 min (Fig. 3F). The effect of norketamine was smaller than that of ketamine at 10–15 min.

Ketamine and norketamine attenuate morphine tolerance more effectively than oxycodone tolerance

In morphine-treated rats, ketamine 10 mg kg^{-1} elicited a 5.2 times larger anti-nociceptive area under the curve (AUC) over 0–150 min compared with oxycodone-treated rats in the tail-flick test (Supplementary Fig. 2A). In the hot-plate test (Supplementary Fig. 2B), the difference was not significant. Norketamine 30 mg kg^{-1} caused greater anti-nociceptive AUCs both in the tail-flick and hot-plate tests (2.3- and 2.0-fold increase, respectively) when administered to morphine-treated rats.

Ketamine and norketamine increase morphine concentrations during chronic morphine treatment

On Day 7, the rats received the same drugs as on Day 6, and tissue samples were obtained at 30 and 90 min after administration. At 30 and 90 min, ketamine 10 mg kg^{-1} and norketamine 30 mg kg^{-1} increased the morphine serum concentrations compared with the vehicle-treated group (Fig. 4A). In the brain (Fig. 4B), the increase in the mean morphine concentration was almost five-fold in the norketamine 30 mg kg^{-1} group (Table 1). Compared with vehicle, the brain-to-serum ratios of morphine did not differ between treatment groups: vehicle 0.32 (0.18), ketamine 10 mg kg^{-1} 0.26 (0.14), and norketamine 30 mg kg^{-1} 0.40 (0.06).

The mean serum morphine-3-glucuronide (M3G) concentration was not affected by ketamine or norketamine (Fig. 4C). However, ketamine and norketamine significantly reduced the serum M3G:morphine metabolic ratio at 90 min (Supplementary Fig. 3).

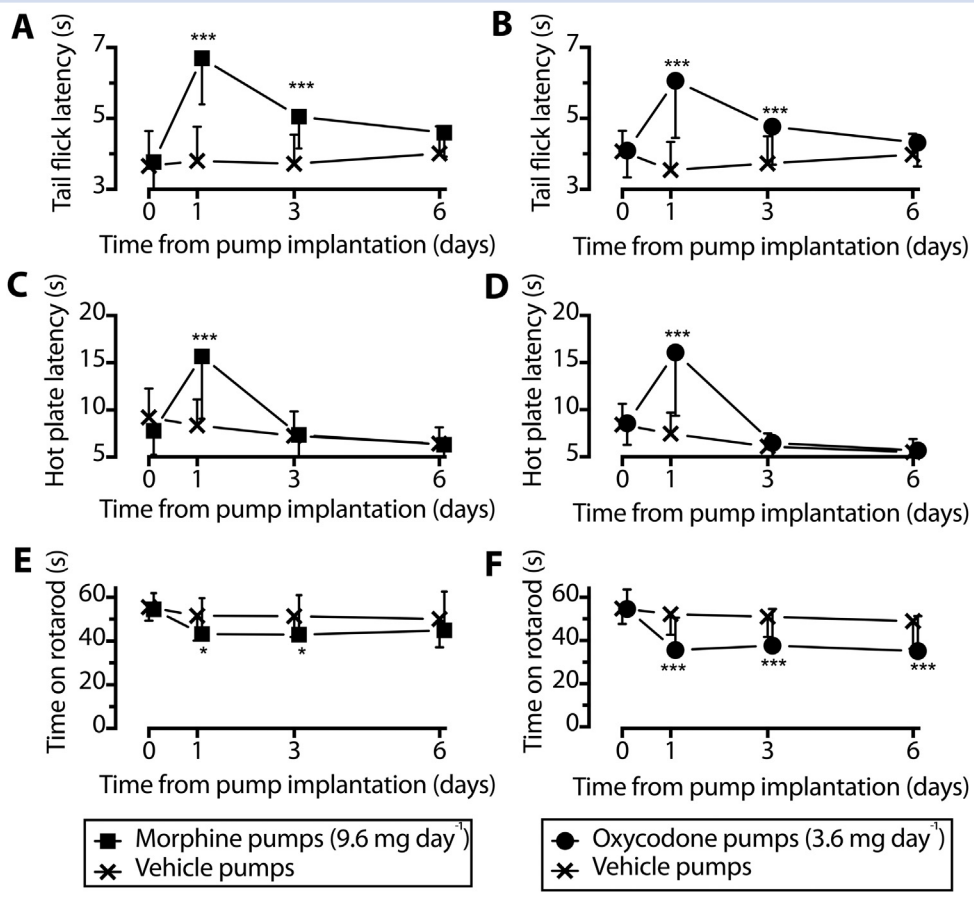

Fig 1. Effects of chronic administration of morphine 9.6 mg day^{-1} and oxycodone 3.6 mg day^{-1} on nociception and motor coordination. Mean (standard deviation) nociceptive latencies for the (A–B) tail-flick and (C–D) hot-plate tests are shown at 1, 3, and 6 days after pump implantation. (E–F) The rotarod test shows effects on motor coordination. Mean raw measurement latencies are shown. ***P<0.001; significantly different from the vehicle group; n=36 in both groups in the morphine experiment; n=30 in both groups in the oxycodone experiment.

Morphine pretreatment increases ketamine and norketamine concentrations

Morphine pretreatment doubled the ketamine concentrations in both serum (Fig. 4D) and brain (Fig. 4E; Table 1) at 30 min. No difference was seen in the brain-to-serum ratio of ketamine between the morphine and vehicle groups [2.0 (0.38) and 2.1 (0.37), respectively]. After ketamine administration, the norketamine concentrations were elevated by morphine pretreatment in serum at 90 min (Fig. 4F), and in the brain at 30 and 90 min (Fig. 4G).

Norketamine was administered at two doses (10 and 30 mg kg^{-1} s.c.). Morphine pretreatment significantly increased the concentration of norketamine in both serum (Fig. 4F) and brain (Fig. 4G; Table 1) at both doses at both time points. No difference was seen in the brain-to-serum ratio of norketamine between the morphine and vehicle groups [0.80 (0.11) and 0.66 (0.11), respectively] at 30 min.

Reversal of oxycodone tolerance by ketamine or norketamine is not associated with changes in drug concentrations

Oxycodone- and vehicle-treated rats received ketamine or norketamine on Day 7. Substantial amounts of oxycodone were found in serum and brain (Fig. 5A and B). Smaller amounts of noroxycodone (Fig. 5C and D) and oxymorphone (Fig. 5E and F) were observed. The concentrations of noroxymorphone in the brain and serum were below the limit of quantification. Ketamine or norketamine did not affect the concentrations of oxycodone (Table 1) or noroxycodone, but both treatments increased the concentrations of oxymorphone in serum and brain. Similar brain and serum concentrations of ketamine and norketamine as in the morphine experiment were observed. However, the oxycodone treatment did not change the ketamine or norketamine concentrations in either serum or brain (Fig. 5G–J; Table 1).

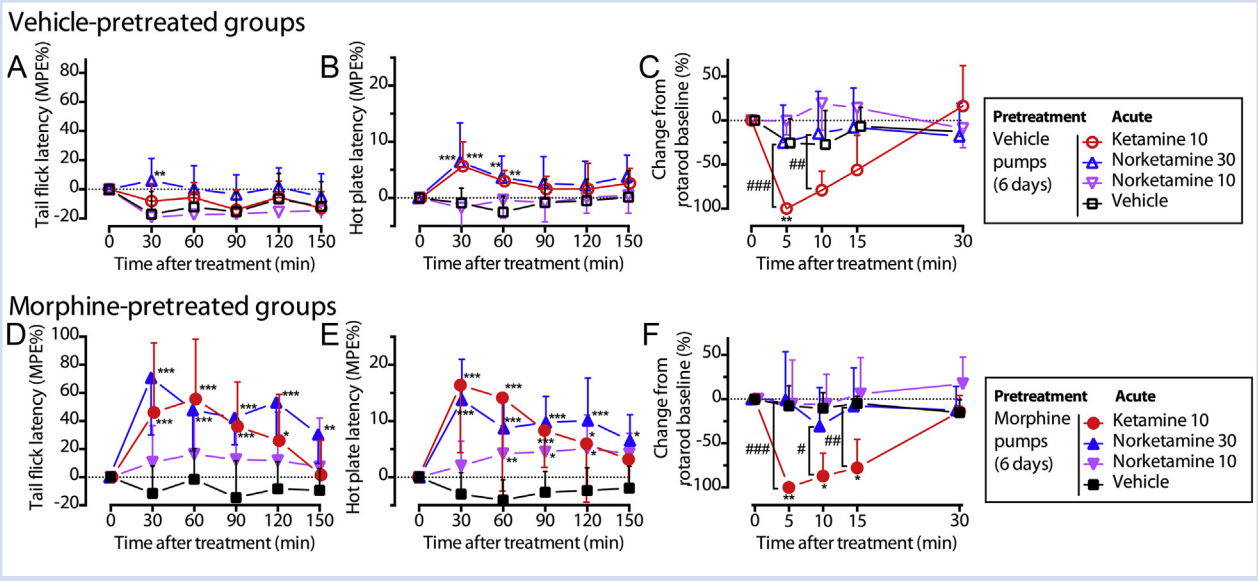

Fig 2. Comparison of the effects of ketamine 10 mg kg^{-1} or norketamine 10 or 30 mg kg^{-1} on the reversal of morphine anti-nociceptive tolerance in rats receiving vehicle or morphine for 6 days. For clarity, the data for the (A–C) vehicle-treated groups and (D–F) morphine-treated groups are shown separately. Nociception was measured up to 150 min after acute drug administration using (A and D) tail-flick and (B and E) hot-plate tests. (C and F) The rotarod test (data shown only up to 30 min) shows effects on motor coordination. For nociceptive tests, the mean (SD) of the MPE% is shown. For the rotarod test, the mean (SD) percentage change from the baseline is shown. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; significantly different from the group that received vehicle acutely. $^{\#}P<0.05$, $^{\#\#\#}P<0.001$; significant difference between the indicated groups. In the nociceptive tests, $n=8$ in all groups, except for the norketamine 10 mg kg^{-1} ($n=12$). In the rotarod test, $n=4$ for all groups. MPE%, percentage of the maximum possible effect; SD, standard deviation.

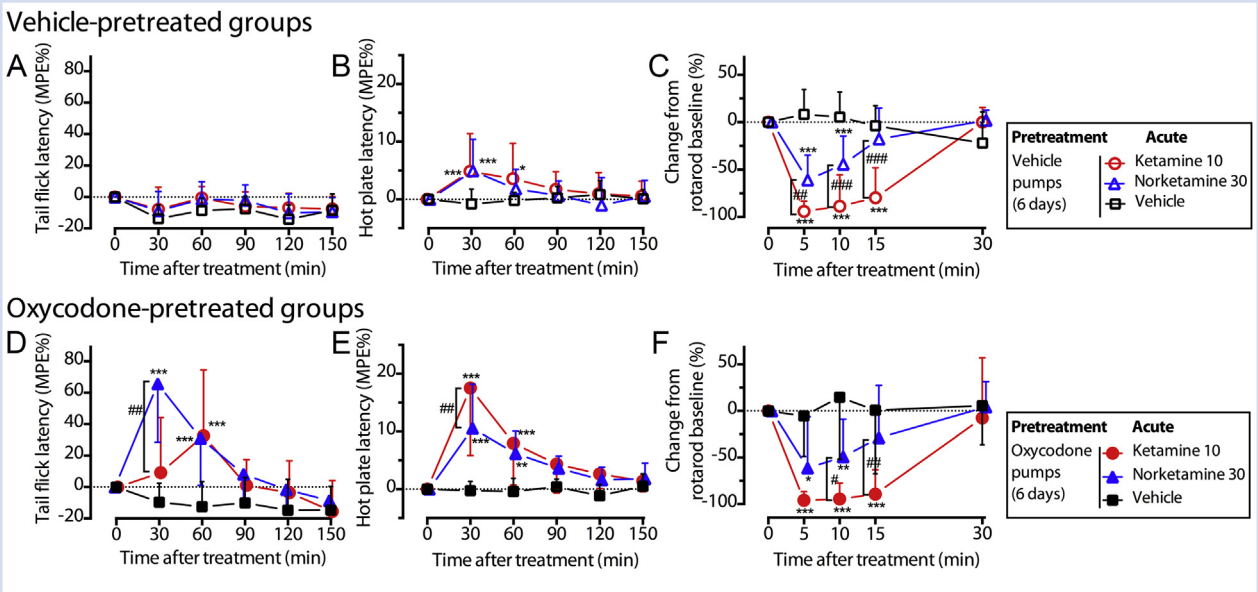

Fig 3. Comparison of the effects of ketamine 10 mg kg^{-1} or norketamine 10 or 30 mg kg^{-1} on the reversal of oxycodone anti-nociceptive tolerance in rats receiving vehicle or oxycodone for 6 days. For clarity, the data for the (A–C) vehicle-treated groups and (D–F) oxycodone-treated groups are shown separately. Nociception was measured up to 150 min after acute drug administration using (A and D) tail-flick and (B and E) hot-plate tests. (C and F) The rotarod test (data shown only up to 30 min) shows effects on motor coordination For nociceptive tests, the mean (SD) of the MPE% is shown. For the rotarod test, the mean (SD) percentage change from the baseline is shown. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; significant differences from the group that received vehicle acutely. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$; significant differences between the indicated groups; $n=10$ in all groups. MPE%, percentage of the maximum possible effect; SD, standard deviation.

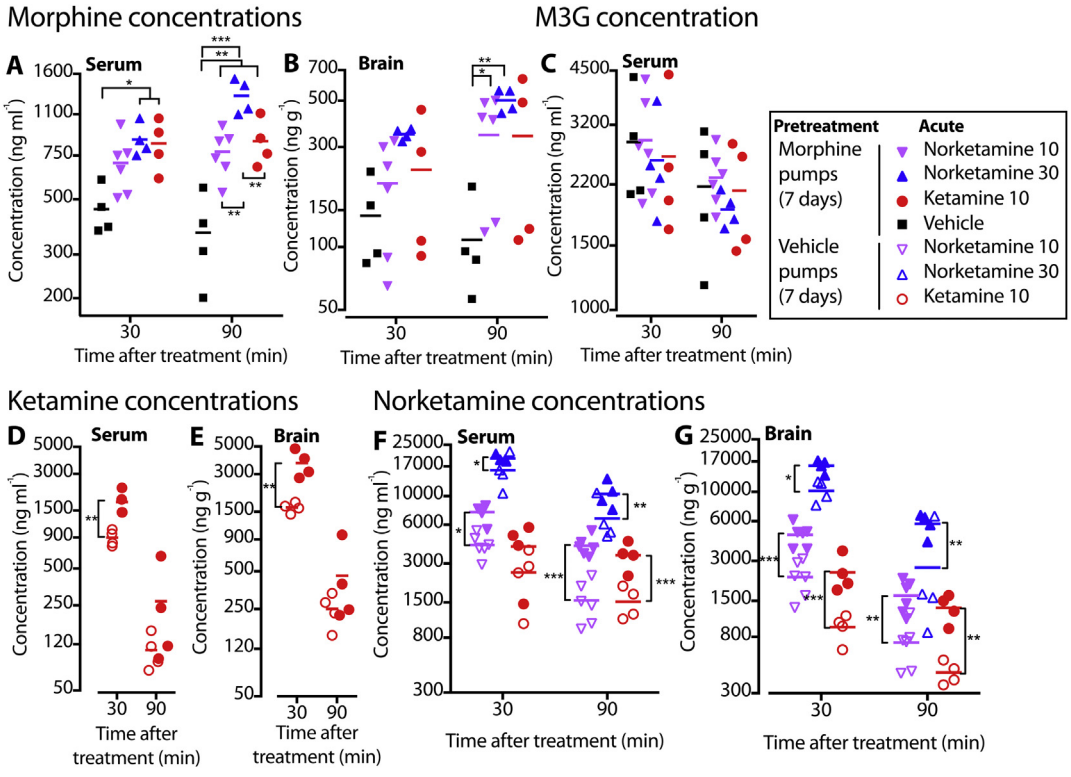

Fig 4. Serum and whole brain concentrations of (A–B) morphine, (D–E) ketamine, and (F–G) norketamine, and (C) serum M3G concentrations in vehicle- or morphine-pretreated rats given ketamine 10 mg kg^{-1} or its metabolite norketamine 10 or 30 mg kg^{-1} on Day 6, and then again on Day 7. Rats were divided into two subgroups for concentration measurements at 30 and 90 min after acute drug administration. Means and the individual measurement points are shown. *P<0.05, **P<0.01, ***P<0.001 between indicated groups with n=4 per measurement point, except in the group receiving norketamine 10 mg kg^{-1} (n=6). KET, ketamine; MO, morphine; M3G, morphine-3-glucuronide; NK, norketamine; VEH, vehicle.

Table 1 Summary of the percentage changes (%) of the mean brain morphine, oxycodone, ketamine, and norketamine concentrations during co-administration compared with the groups that received only a single active drug (morphine, oxycodone, ketamine, or norketamine) without an interacting drug. n/a, not available (oxycodone-treated rats did not receive norketamine 10 mg kg^{-1})

Effects of acute ketamine or norketamine administration on brain concentrations of morphine and oxycodone							
Acute treatment		**Ketamine 10 mg kg^{-1}**		**Norketamine 30 mg kg^{-1}**		**Norketamine 10 mg kg^{-1}**	
Time point (min)		**30**	**90**	**30**	**90**	**30**	**90**
Change in brain concentration (%)	Morphine	+66	+210	+150	+360**	+43**	+220*
	Oxycodone	+16	−5	+13	+13	n/a	n/a

Effects of morphine or oxycodone pretreatment on brain ketamine and norketamine concentrations					
Chronic pretreatment		**Morphine**		**Oxycodone**	
Time point (min)		**30**	**90**	**30**	**90**
Change in brain concentration (%)	Ketamine (after 10 mg kg^{-1})	+130**	+84	−2	−9
	Norketamine (after 30 mg kg^{-1})	+55*	+110**	+25	−20
	Norketamine (after 10 mg kg^{-1})	+110***	+130**	n/a	n/a

*P<0.05, **P<0.01, ***P<0.001.

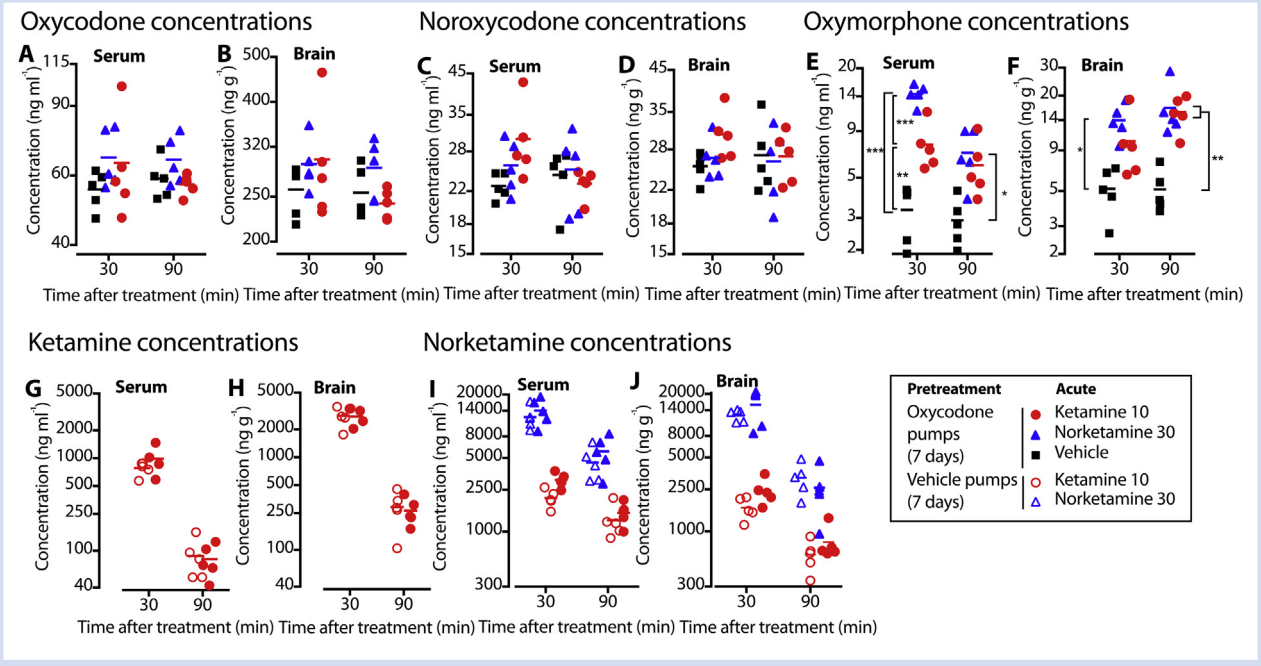

Fig 5. Serum and whole brain concentrations of (A–B) oxycodone, (C–D) noroxycodone, (E–F) oxymorphone, (G–H) ketamine, and (I–J) norketamine in vehicle- or oxycodone-pretreated rats given ketamine 10 mg kg^{-1} or its metabolite norketamine 30 mg kg^{-1} on Day 6, and then again on Day 7. Rats were divided into two subgroups for concentration measurements at 30 and 90 min after acute drug administration. Means and the individual measurement points are shown. The serum and brain concentrations of noroxymorphone were below the limit of quantification in all groups. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ between indicated groups; $n=5$ per measurement point. Ket, ketamine; Norket, norketamine; Ox, oxycodone; Veh, vehicle.

The synergistic anti-nociceptive effect between oxycodone and ketamine or norketamine is not associated with drug concentration changes

Drug-naïve rats received oxycodone at −15 min and ketamine, norketamine, or vehicle. In the tail-flick test (Supplementary Fig. 4A), ketamine augmented the effect of oxycodone, whereas the effect of norketamine was not significant. In the hot-plate test (Supplementary Fig. 4B), ketamine had a pronounced effect on oxycodone anti-nociception lasting up to 60 min, whereas the effect of norketamine on oxycodone anti-nociception was significant only at the 30 min time point. Co-administration of oxycodone and ketamine, but not norketamine, disturbed the motor coordination at 5–15 min after administration (Supplementary Fig. 4C). After a week of washout, after a similar drug treatment, neither ketamine nor norketamine changed the concentrations of oxycodone in serum (Supplementary Fig. 4D) or brain (Supplementary Fig. 4E) at 30 min.

Discussion

The norketamine- and ketamine-induced reversal of morphine tolerance was much greater than that of oxycodone tolerance. In particular, the attenuation of tolerance was markedly longer in morphine-tolerant rats. Both ketamine and norketamine induced long-lasting increases in brain and serum concentrations of morphine when administered to rats under chronic morphine treatment. In sharp contrast, the oxycodone concentrations were not increased. Pretreatment with morphine, but not oxycodone, increased the brain and serum concentrations of ketamine and norketamine. Norketamine attenuated morphine tolerance in a similar manner as ketamine at a three times larger dose with significantly less adverse effects.

Ketamine attenuated morphine tolerance in line with previous research.[3,5,19] Interestingly, a three-fold higher dose of norketamine also reversed the morphine tolerance in a similar fashion. The estimated NMDA receptor occupancy based on drug concentrations and assumed receptor binding affinity (ketamine has about four times higher affinity than norketamine[6–9]) in the brain was relatively similar at 30 min, but at 90 min it was higher after norketamine administration, a finding that could explain the longer effect of norketamine. These data demonstrate that norketamine easily penetrates the brain. Interestingly, despite the high brain concentrations and the reversal of morphine tolerance, norketamine did not cause observable motor deficits in the rotarod test, an adverse effect clearly observed shortly after the administration of ketamine. This may be explained by a slower central nervous system penetration of norketamine because of the higher hydrophilicity of norketamine compared with ketamine.[7]

Surprisingly, oxycodone tolerance was attenuated to a lesser extent by ketamine and norketamine (Fig. 3). To the best of our knowledge, this is the first study suggesting that the effect of ketamine may be smaller in oxycodone than morphine tolerance. Various pharmacodynamic differences, such as biased agonism at the μ-opioid receptor, could be

involved. Interestingly, NMDA receptor antagonism has been proposed to prevent μ-opioid receptor endocytosis,[20] and β-arrestin2-mediated functional desensitization of the receptor may be more pronounced during morphine than oxycodone treatment.[21] However, NMDA receptor-mediated pathways leading to μ-opioid receptor desensitization have not been determined,[22] and the effects of NMDA receptor antagonism on μ-opioid receptor kinetics during treatment with different opioids remain to be studied. As shown in the present study, in addition to potential pharmacodynamic differences, pharmacokinetic interactions also need to be considered in the attenuation of opioid tolerance by ketamine.

We have shown previously that morphine and ketamine have pharmacokinetic interactions after chronic morphine administration.[5] This study shows similar findings with morphine and norketamine. Both ketamine and norketamine increased the morphine brain concentration three- to five-fold 90 min after administration (Table 1). However, increased morphine concentration in the brain does not alone explain the attenuation of morphine tolerance: at the 90 min measurement point, norketamine 10 mg kg^{-1} increased the brain concentration of morphine in the same manner as ketamine 10 mg kg^{-1}, but only ketamine markedly attenuated tolerance. In rats receiving chronic morphine, both norketamine and ketamine concentrations were significantly increased. Thus, the increased brain exposure to all three substances is likely to contribute to the increased anti-nociception in morphine-tolerant animals. Similar pharmacokinetic interactions were not seen in oxycodone-treated animals. As ketamine and norketamine only transiently reversed oxycodone anti-nociceptive tolerance, the lack of pharmacokinetic interactions seen with morphine could partially explain the difference in behavioural data between morphine and oxycodone.

The brain-to-serum ratio of morphine was not changed by acute administration of ketamine or norketamine, and chronic morphine treatment did not change the brain-to-serum ratio of ketamine or norketamine. This suggests that the changes in the brain drug concentrations are not because of changes at the blood–brain barrier. Morphine is extensively metabolized by uridine diphosphate-glucuronosyltransferase (UGT) 2B7 in humans[23] and 2b1 in rats,[24] whereas oxycodone is metabolized by cytochrome P450 (CYP) enzymes.[25] Neither ketamine nor norketamine affected the oxycodone concentrations during acute or chronic oxycodone administration, even though some of the same CYP enzymes are involved in the metabolism of the drugs.[1,25] The finding that morphine concentrations, but not oxycodone concentrations, were increased by ketamine and norketamine would suggest that the UGT system rather than the CYP system could be involved.

Both *in vitro* and *in vivo* evidence supports the hypothesis that inhibition of UGT by ketamine and norketamine may inhibit morphine metabolism, leading to the demonstrated increased morphine concentrations. Clinically relevant concentrations of ketamine have been shown to inhibit the human UGT2B7 enzyme-mediated metabolism of morphine to its glucuronides.[26,27] Here, both ketamine and norketamine decreased the M3G:morphine serum metabolic ratio. Moreover, acute single-dose co-administration of ketamine decreased the hepatic M3G:morphine metabolic ratio 3.8-fold in chronic morphine administration,[5] demonstrating the inhibition by ketamine of morphine metabolism *in vivo*. Thus, the dramatically increased morphine concentrations after ketamine and norketamine co-administration are most likely caused by ketamine- and norketamine-mediated inhibition of morphine metabolism.

In the largest randomized study on ketamine as an adjuvant to opioids in cancer pain, the number needed for harm for ketamine was only six.[16] These adverse events could be in part attributable to increased morphine concentrations. The decreased clearance and increased elimination half-life may result in higher steady-state plasma concentrations and overdosing. Thus, to detect potential pharmacokinetic interactions, morphine concentrations should be monitored when initiating ketamine treatments to high-dose morphine patients.

In conclusion, ketamine and norketamine effectively attenuated morphine tolerance. Oxycodone tolerance was less affected. This difference may be partly attributable to the inhibition of morphine metabolism by ketamine and norketamine, leading to three- to five-fold increases in brain morphine concentrations. The co-administration of oxycodone with ketamine or norketamine did not show pharmacokinetic interactions. The differences between opioids should be considered in randomized controlled trials assessing the combined use of ketamine and opioids. Norketamine should be further studied in the treatment of opioid tolerance, as it attenuated morphine tolerance with minor adverse effects.

Authors' contributions

Conception and design, acquisition of data, analysis and interpretation of data, writing the manuscript, and approval of the final manuscript: T.L., E.K., P.R.
Acquisition of data, analysis and interpretation of data, and approval of the final manuscript: M.N.
Conception and design, analysis and interpretation of data, writing the manuscript, and approval of the final manuscript: E.K.

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Declaration of interest

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bja.2017.11.081.

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