Abstract

The continuous rise in opioid overdoses in the United States is predominantly driven by very potent synthetic opioids, mostly fentanyl and its derivatives (fentanyls). Although naloxone (NLX) has been shown to effectively reverse overdoses by conventional opioids, there may be a need for higher or repeated doses of NLX to revert overdoses from highly potent fentanyls. Here, we used positron emission tomography (PET) to assess NLX's dose-dependence on both its rate of displacement of $[^{11}\text{C}]$carfentanil ($[^{11}\text{C}]$CFN) binding and its duration of mu opioid receptor (MOR) occupancy in the male rat brain. We showed that clinically relevant doses of intravenously (IV) administered NLX (0.035mg/kg, Human Equivalent Dose(HED) 0.4mg; 0.17mg/kg, HED 2mg) rapidly displaced the specific binding of $[^{11}\text{C}]$CFN in thalamus in a dose-dependent manner. Brain MOR occupancy by IV NLX was greater than 90% at 5 minutes after NLX administration for both doses, but only 50% occupancy remained at 27.3 min and at 85 min after 0.035mg/Kg and 0.17 mg/Kg NLX, respectively. This indicates that the duration of NLX occupancy at MORs is short-lived. Overall, these results show that clinically relevant doses of IV NLX can promptly displace fentanyls at brain MORs, but that repeated or higher NLX doses may be required to prevent re-narcotization following overdoses with long-acting fentanyls.

Introduction

Over the past 5 years, the opioid overdose epidemic in the United States [1] has been exacerbated by the rise in illicit synthetic opioids such as fentanyl (1) and its derivatives (fentanyls, Fig. 1) [2–4]. Indeed, in the 12 months preceding March 2021 alone, the CDC estimated a total of 63,075 synthetic opioid overdose deaths, a 54.5% increase from the previous year [5]. This persistent increase in fentanyls-related overdose deaths reflects their highly rewarding effects, potency in inducing respiratory depression, and their widespread availability driven by its ease of production and distribution [6–8]. Such challenges are, thus, forcing health care providers to reconsider their strategies for treating overdoses caused by synthetic opioids [9–11].

Naloxone (3, NLX) is the most effective clinically available medication to reverse opioid-induced overdoses (Fig. 1) [6, 12]. It is a potent antagonist ($K_d=0.73\text{ nM}$, [13]) of the mu-opioid receptor (MOR), which is the target underlying opioid induced analgesia, reward and respiratory depression [14, 15]. Parenteral (0.4 mg, 2 mg) and intranasal (IN, 2 mg, 4 mg) NLX formulations are available over the counter in most states for opioid overdose reversal. However, there are growing concerns that those options may be ineffective in reversing overdoses of highly potent synthetic opioids such as fentanyl ($K_i=0.39\text{ nM}$) [16] and carfentanil (2, CFN, $K_d=0.08\text{ nM}$) [17]. Additionally, the relatively short duration of NLX’s action may be insufficient for preventing re-narcotization after overdose with the longer-lasting fentanyls. In fact, Tomassoni et al. noted, “Some patients required doses of the opioid antidote naloxone exceeding 4 mg (usual initial dose = 0.1–0.2 mg intravenously), and several patients who were alert after receiving naloxone subsequently developed respiratory failure” [18]. Multiple NLX injections are sometimes required to maintain adequate breathing following re-narcotization after initial NLX treatment [19]. Thus,
a better understanding of NLX's blockade of the MOR over time would help to improve our clinical guidelines regarding NLX administration and dosing for overdose reversal from fentanyl.

Positron emission tomography (PET) and the MOR radioligand, $[^{11}\text{C}]$CFN have been used to measure blockade of MOR by NLX non-invasively in humans [20] and in non-human primates [21]. Most $[^{11}\text{C}]$CFN PET studies have assessed receptor occupancy (RO) within 10 min after an acute NLX dose for various administration methods [19, 22–30]. In particular, two relevant clinical reports have been published on NLX's clearance rate from MORs. One used a dual coincidence detector system to compare intravenous (IV) NLX with IV nalmefene [31]; the other used PET to obtain RO at two time points, after intranasal (IN) NLX [32]. In the current study, we used PET to measure the displacement of $[^{11}\text{C}]$CFN binding by IV NLX in the rodent brain. Subsequently we obtained RO at multiple time points to characterize the clearance profile over 2.5 hrs following IV NLX. For both set of experiments, we compared two clinically relevant IV NLX doses (0.035 and 0.17 mg/kg), which correspond to human equivalent doses (HED) of 0.4 mg and 2.0 mg respectively.

**Results**

$[^{11}\text{C}]$CFN radiosynthesis and administration

Averaged radiochemical yield and molar activity at the end of the bombardment were 50.0 ± 12% and 1172 ± 938 GBq/µmol, respectively. The average molar activity and injected CFN mass at the time of $[^{11}\text{C}]$CFN injection were 226.4 ± 162.4 GBq/µmol and 60.4±55 ng/kg, respectively. The HPLC analysis confirmed high radiochemical purity (>99%).

$[^{11}\text{C}]$CFN binding displacement by intravenous NLX

In $[^{11}\text{C}]$CFN PET scans, thalamus showed high uptake and specific binding and was used as the region of interest (ROI) to quantify specific binding and RO by NLX [33]. Cerebellum showed fast $[^{11}\text{C}]$CFN clearance and was used as a reference region (SI Fig. 1A). Averaged clearance half-time ($t_{1/2}$) from peak was 41.84 min for thalamus and 7.33 min for cerebellum. IV NLX pretreatment at 5 min prior to $[^{11}\text{C}]$CFN injection reduced $[^{11}\text{C}]$CFN uptake in thalamus to the level of uptake seen in cerebellum (SI Fig. 2). This provides preclinical evidence suggesting that currently approved doses of IV NLX can abolish specific binding of $[^{11}\text{C}]$CFN, and are likely at peak levels, to temporarily occupy nearly all MORs in brain.

To characterize NLX's displacement of $[^{11}\text{C}]$CFN at MORs, NLX administration was given at 15 min after $[^{11}\text{C}]$CFN injection. Both doses of NLX gradually diminished $[^{11}\text{C}]$CFN binding in the thalamus to the level observed in the cerebellum (reference region), but the higher dose displaced it faster than the lower one (Fig. 2 and Fig. 3). Time-activity curves of $[^{11}\text{C}]$CFN in the thalamus prior to NLX injection did not differ statistically between the control and the NLX treatment groups (Student t-test p= 0.14 for 0.17 mg/kg; p=0.31 for 0.035 mg/kg) (Fig. 2B). After the IV NLX challenge, the time to reach equivalent levels of $[^{11}\text{C}]$CFN uptake in the thalamus and cerebellum (ratio of standard uptake value, SUVr =1) was 61.76 ±
9.49 min for the lower NLX dose (0.035 mg/kg) and 34.42 ± 3.56 min for the higher dose (0.17mg/kg), and these differences between the two doses was significant (p=0.0095, student t-test) (Fig. 2C). Estimation of the absolute gradient change after NLX injection [34], showed that NLX’s displacement of $[^{11}]$CFN was significantly faster for the 0.17 mg/kg NLX dose than for the 0.035 mg/kg dose (p=0.002, student t-test, n=3) (Fig. 2D).

**Duration of receptor occupancy after pretreatment with IV NLX**

Rats were pretreated with IV NLX (0.035 mg/kg, 0.17mg/kg) at various time points before $[^{11}]$CFN injection. While there were no differences in $[^{11}]$CFN uptake in the cerebellum for any time points, the uptake of $[^{11}]$CFN in the thalamus differed significantly between the various time points of NLX pretreatment showing a gradual recovery towards baseline over two hours. The non-displaceable binding potentials ($BP_{ND}$) of $[^{11}]$CFN at baseline (n=8) and at various NLX pretreatment times are summarized in Supplemental Table 1. The higher dose of IV NLX (0.17 mg/kg) blocked $[^{11}]$CFN binding in the thalamus longer than the lower dose (0.035 mg/kg). Specifically, 90 min after IV NLX injection, the averaged RO of 0.17 mg/kg NLX was significantly higher than that of 0.035 mg/kg NLX (60% vs 12% RO, p = 0.02) (Fig. 4). Consistently, the clearance half-time of RO for the thalamus was 27.3 min for the 0.035 mg/kg NLX and 85 min for 0.017 mg/kg NLX (Fig. 5).

**Pharmacokinetics assessment of IV NLX**

The measured peak plasma NLX concentration differed significantly between the two doses (0.17 mg/kg: 77 ng/ml; 0.035 mg/kg: 1.7 ng/ml; Student t-test, p=0.01) (Fig. 5). Table 1 shows the estimated pharmacokinetic data for IV NLX in plasma based on a non-compartmental analysis. The average elimination rate of IV NLX ($K_e$) was 16 min for 0.17 mg/kg and 7.3 min for 0.035 mg/kg NLX, respectively.

**Correlation between receptor occupancy and plasma naloxone concentration**

NLX plasma concentration decreased rapidly for the two doses of IV NLX, but lasted longer for the higher dose (SI Fig. 3). The plasma NLX levels necessary to achieve half-maximal receptor occupancy ($EC_{50}$) were estimated to be 0.2 ng/mL as assessed by fitting the association between NLX ROS and plasma NLX concentrations (SI Fig. 4). NLX RO levels plateau at 90% for NLX plasma concentration higher than 1.5 ng/ml.

| Dose (mg/kg) | Half-life (min) | Tmax (min) | Cmax (ng/mL) | AUC (0-inf) |
|--------------|----------------|------------|--------------|-------------|
| 0.035 (n=3)  | 7.3 (3.5)      | 6.8 (3.3)  | 12.4 (1.7)   | 53.2        |
| 0.17 (n=3)   | 16 (5.8)       | 15.0 (5.0) | 50.5 (1)     | 489.6       |
Discussion

Highly potent fentanyl derivatives are the main contributors to the steep rise in opioid overdose mortality. To cope with the challenges in reversing overdoses with potent fentanyls, the Food and Drug Administration (FDA) recently approved a high dose of IN NLX (8 mg, [35]) and a 5 mg NLX injection dose [36]. Balancing the risks and benefits of increasing NLX doses used for opioid reversal is a topic of clinical interest [37]. Thus, understanding the relationship between NLX’s dose and its onset and duration of MOR blockade will play a key role in developing proper guidelines for fentanyl overdose reversal.

In the current study, we used clinically relevant doses of IV NLX to characterize their efficacy in displacing \(^{11}\text{C}\)CFN in the male rat brain. Moreover, we quantified the rate and duration of MOR occupancy by IV NLX over time. We showed that \(^{11}\text{C}\)CFN clearance rate by IV NLX was dose-dependent with the higher dose displacing CFN more quickly than the lower dose. Specifically, the clearance time to reach half of \(^{11}\text{C}\)CFN specific binding in the thalamus for the 0.17 mg/kg NLX (HED 2 mg) dose was 21.74 min, significantly faster than 27.05 min for the 0.035 mg/kg NLX (HED 0.4mg) dose. This result would therefore suggest that higher NLX doses are likely to exhibit faster onset of action in overdose reversal settings than lower NLX doses.

The higher NLX dose also resulted in a longer duration of MOR RO than the lower NLX dose. We built up RO profiles by performing \(^{11}\text{C}\)CFN PET studies at multiple time points after NLX administration separately for each of the two doses. Half time of RO clearance was 27 min for the 0.035 mg/kg NLX dose whereas it was 85 min for the 0.17 mg/Kg dose, showing that the duration of MOR blockage by NLX is dose dependent. The duration of greater than 80% MOR blockade for the 0.17 mg/kg dose was five times longer compared to that for the 0.035 kg/mg dose. As shown in Figure 3, RO of less than 30% occurred within 50 min for the lower dose (0.035 mg/kg) whereas it occurred at 100 min for the higher dose (0.17 mg/kg). The fast RO clearance observed with IV NLX could explain why multiple NLX doses are often required to prevent re-narcotization following overdoses with fentanylls [38].

In a separate group of rats, NLX pharmacokinetics were measured in plasma for the two NLX doses and to correlate them with levels of RO, we estimated that the plasma NLX concentration for half-maximal receptor occupancy (EC\(_{50}\)) was low (0.2 ng/ml). However, it is likely that the NLX concentration in brain was much higher than in plasma (SI Fig. 3) due to NLX’s high lipophilicity and the short-lasting peak in plasma after IV administration. Future studies are required to assess the relationship between plasma and brain NLX levels and to ascertain what level of RO is needed to restore and sustain proper breathing following fentanyl overdose.

Previously, Kim et al. measured \(^{11}\text{C}\)CFN uptake in the human brain at four time points over 9 hours using a dual coincidence detector system. They reported that the clearance half-time of RO by IV NLX (2 mg) was 2 ± 1.6 hr after administration [31]. These data, in conjunction with our own, suggest that 2 mg IV NLX might be inadequate for preventing re-narcotization after overdose with long half-life fentanylls (e.g., CFN T\(_{1/2}\) = 7.7 hr). Similarly, Johansson et al. [32] used PET to measure the specific binding of \(^{11}\text{C}\)CFN
in healthy human brains at two separate time intervals (0-60 min, 300-360 min) after administration of 2 mg IN NLX. They found that the clearance half-time of RO occurred 2-3 hours after NLX administration and that the duration of NLX's RO at MORs was significantly longer for the 4 mg dose than the 2 mg dose. Thus, both clinical and preclinical studies provide evidence that may justify the use of higher doses of NLX, thereby corroborating the FDA's recent decision to approve higher NLX formulations [35, 36].

Despite these conclusions, there are serious drawbacks for using higher dose of NLX, namely, NLX-precipitated withdrawal symptoms. Likewise, the use of potent MOR antagonists with a longer duration of action, such as naltrexone and nalmefene, has been proposed [39], but concerns of protracted opioid withdrawal need to be considered. In this respect, it would be valuable to determine the minimal levels of RO needed to sustain normal breathing and cardiovascular function following an overdose.

Limitations

Our study is limited by species differences in both the NLX pharmacokinetics under anesthesia, and pharmacodynamics of opioid induced respiratory depression. Since the acquisition time for a single PET scan requires at least approximately 1 hr, rapid RO change could not be accurately measured. Additionally, it was also challenging to predict RO with plasma NLX concentrations over time because NLX's clearance rate in plasma is very fast. Although our displacement studies showed prompt and very effective displacement of $[^{11}\text{C}]$CFN binding by IV NLX, these experiments used tracer doses of CFN and pharmacological doses of fentanyl may exhibit slower displacement rate. Our study, as well as prior studies, evaluated NLX's pharmacokinetics of $[^{11}\text{C}]$CFN displacement and RO in animals or individuals who were physiologically stable, whereas opioid overdoses subjects may have complications in cardiovascular function, which could jeopardize NLX bioavailability and delivery to the brain. Finally, after surveying all previous NLX RO studies, we point out that there is little information on what level of RO is effective for opioid overdose reversal, which would be valuable to provide clear guidelines for clinical therapeutics.

Conclusion

Using two clinically relevant doses of IV NLX, we documented fast and effective displacement of $[^{11}\text{C}]$CFN binding but short lasting MOR occupancy in the rodent brain. The effects were dose dependent such that a higher dose of NLX displaced CFN faster and had longer duration of MOR blockade. Our results indicate that higher initial doses of NLX could more quickly revert overdose and that repeated doses could help to prevent renarcotization from these long-acting fentanyl.

Materials And Methods

General

The precursor for $[^{11}\text{C}]$CFN, Desmethylcarfentanil acid, was purchased from American Biochemicals (College Station, TX, USA). All other chemicals were purchased from Millipore Sigma and used without
any further purification. No-carrier-added $^{[11]}$C$\text{CO}_2$ was generated by nuclear reaction $^{14}$N(p,α)$^{[11]}$C, bombarding a nitrogen gas target containing 1% oxygen with a proton beam using a cyclotron (PETrace, GE). Conversion to $^{[11]}$CCH$_3$I and $^{[11]}$Cmethylation were performed using FX-Mel and FX-M automated synthesizers (GE Healthcare, Chicago, IL USA), respectively. Semi-preparative high performance liquid chromatography (HPLC) was used to purify a crude $^{[11]}$C$\text{CFN}$ mixture with a monolithic column (10x100 mm, Onyx Monolithic C18, Phenomenex; flow rate, 5 mL/min; eluent, 0.01M phosphate buffer/ethanol=51.5/48.5; pH = 7.2-7.4; UV wavelength, 218 nm; retention time, 8 min). For quality control, analytical HPLC analysis was performed using an Agilent 1100 system equipped with a ZORBAX Eclipse XDB C18 column (4.6x150mm, Agilent, Santa Clara, CA), monitoring for absorbance at 218 nm and radioactivity using a flow count radioactivity detector (Carroll Ramsey and Associates, Fort Collins, CO, USA). $^{[11]}$C$\text{CFN}$ was eluted at a flow rate of 1.0 mL/min (retention time, 5 min) with an isocratic solvent mixture (water/acetonitrile, 60/40) containing trifluoroacetic acid (0.1%).

All rat studies were approved by the Clinical Center Animal Care and Use Committee of National Institutes of Health (protocol number, NIAAA 19-01) and complied with the Guide for the Care and Use of Laboratory Animals. $^{[11]}$C$\text{CFN}$ PET studies were performed in male Long Evans rats (294.7 ± 69.6 g, Charles River Laboratories) using a small animal PET scanner (MicroPET Focus 220, Siemens). Animals were anesthetized with isoflurane (Forane, Baxter Healthcare) using an anesthesia machine (SurgiVet VaporStick, Smiths Medical) and vaporizer (SurgiVet 100 Series, Smiths Medical). Vitals (heart rate, respiratory rate, spO$_2$, and temperature) were monitored using a pulse oximeter and heart rate monitor (MouseSTAT, Kent Scientific). A heat lamp was used to maintain body temperature (Model# 51152, Brandt Industries). Tubing for catheters (BTPE-10 for infusion, BTPU-27 for blood withdrawal) and other surgical materials were obtained from Instech Laboratories. Bolus $^{[11]}$C$\text{CFN}$ injections were performed using a syringe pump (PHD 2000, Harvard Apparatus), while bolus plus constant infusion (B/CI) were performed using a programmable pump (Pump 11 Elite, Harvard Apparatus). Blood plasma was obtained by centrifugation (MiniSpin, Eppendorf). The study is in accordance with ARRIVE guidelines.

Synthesis of $^{[11]}$C$\text{Carfentanil (}[^{11}\text{C}]2)$

$^{[11]}$C$\text{CFN}$ was synthesized according to the reported procedure with minor modifications [40, 41]. Briefly, anhydrous DMSO (200 µL) solution containing desmethylcarfentanil acid (1 mg, 2.64 µmol), Cs$_2$CO$_3$ (2 mg, 6.14 µmol) was vortexed for 1 min. After $^{[11]}$Cmethyl iodide was transferred in a stream of helium at room temperature, the reaction mixture was heated at 120°C for 3 min. Crude mixture was purified with semi-preparative HPLC. The collected portion of $^{[11]}$C$\text{CFN}$ was adjusted to contain less than 10% ethanol content for injections.

Rodent PET Studies

Anesthesia in rats were initially induced with isoflurane (5.0%) in oxygen for 5 min and then was maintained at a lower level of isoflurane (1.5-2.5%), monitoring vitals throughout the experiments.
Catheters were placed in the left femoral vein for $^{11}$C CFN injection. For displacement studies, $^{11}$C CFN was administered as a bolus (1 min), followed by IV NLX 15 min later. For RO studies, $^{11}$C CFN was administered via a B/Cl method ($K_{bol}$ = 80 min) that lasted the entire duration of each scan. Before radiotracer injection, rats were pretreated with IV NLX at selected time points (0.035 mg/kg: 20, 40, 60, 87, 180 min; 0.17 mg/kg: 10, 15, 40, 60, 90, 110, 210 min). List-mode data was acquired over 90 min after a 10 min transmission scan with a Co-57 point source for attenuation correction. PET data was reconstructed into 22 frames (6 x 20 sec, 5 x 60 sec, 4 x 120 sec, 3 x 300 sec, 3 x 600 sec, and 1 x 1200 sec) using filtered back-projection. The average activity injected was 14.0 ± 8.6 MBq and the average CFN mass injected was 60.4 ± 55 ng/kg.

PET imaging processing and tracer kinetic analysis

Time-activity curves were obtained as standard uptake value (SUV, g/mL) using PMOD (3.807). Two regions of interest (ROIs) were analyzed for $^{11}$C CFN uptake: the thalamus due to its high concentration of MORs [42] and high specific binding, and the cerebellum, which was used as a reference region mostly devoid of specific binding [43].

The ROI template was drawn using anatomical information extracted from a $^{18}$F FDG PET scan obtained for this purpose following a $^{11}$C CFN scan in one rat. ROIs were drawn in the cerebellum and thalamus, avoiding border regions, and were applied to generate time activity curves.

SUVr was calculated for each frame as the ratio between thalamic and cerebellar SUVs. For RO studies, $BP_{ND}$ was obtained using the B/Cl method to achieve constant radioactivity levels in the ROIs and in the reference region [44, 45]. Once equilibrium is achieved [46], the binding potential ($BP_{ND}$) was calculated directly from the concentration ratio of thalamus to cerebellum (15-40 min). The value of $BP_{ND}$ can be computed using $BP_{ND} = C_{thalamus}/C_{cerebellum} − 1$. Receptor occupancy [RO(%)] was calculated using Equation 1 [47]:

$$RO(\%) = 100 \times \frac{Baseline(BP_{ND}) - Post\_drug(BP_{ND})}{Baseline(BP_{ND})} - 1$$

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Plasma pharmacokinetics assessment of IV NLX

To determine plasma concentrations of NLX over time, a NLX bolus was administered to 6 rats (n=3, 0.035 mg/kg; n=3, 0.17 mg/kg) via penile vein, and arterial whole blood samples (250 uL) were collected at 0, 1, 3, 5, 10, 15, 30, 45, 60, and 90 min after NLX injection. Each blood sample was centrifuged at 14,500 RPM for 3 min to give each plasma sample, followed by immediate freezing on dry ice until stored at -80°C. Plasma NLX concentration was determined using LC-MS/MS (Bioanalytical Shared Resource Laboratory, Virginia Commonwealth University School of Pharmacy), with a detection limit of NLX was 1
ng/mL. Pharmacokinetics parameters were estimated by non-compartmental analysis and plasma curves were fitted using two exponential clearance model.

Given the plasma concentration and the $K_d$ values for the MOR, RO was calculated according to the reaction kinetics between a MOR and NLX, as follows [48]:

$$\text{Occupancy}(\%) = \frac{C_u}{C_u + K_d}$$

**Declarations**

**Data Availability**

The datasets analysed during the current study are available from the corresponding author on reasonable request.

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**Author contributions**

Y.K, K.O, A.K, J.R. J.S.F, N.D.V, and S.W.K mainly wrote and revise the manuscript. Y.K, K.O, A.K, and S.W.K analyzed data, and created the figures and tables to the document. W.Z S.M.E, T.S, M.G, G.B and S.W.K conducted radiosynthesis. J.R, T.P, N.B, and J.S.L performed PET experiments. Full experimental design was from NDV and SWK.

**Competing interests**

The authors declare no competing interests.

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**Figures**
Figure 1

Structures of fentanyls and naloxone. Each arrow represents positions which have been abundantly reported to generate illegal fentanyls via chemical modification with various substituents.

Fentanyls
1, $R_1 = H$, fentanyl
2, $R_1 = CO_2CH_3$, carfentanil
3, naloxone
Figure 2

$[^{11}\text{C}]$CFN displacement study with two doses of IV NLX (baseline, n=3; 0.035 mg/kg, n=3; 0.17 mg/kg, n=3). Averaged time-activity curves in the cerebellum (A) and thalamus (B) were generated in standard uptake value (SUV, g/mL). Averaged SUVs of thalamus to cerebellum ratios (SUVr) for baseline and the two NLX doses (C). Dose-dependent displacement rate was expressed as absolute gradient change after NLX post-treatment (D). NLX was administered at 15 min after $[^{11}\text{C}]$CFN injection.
Figure 3

Averaged PET brain images of \[^{11}\text{C}]\text{CFN for baseline and the NLX treatments (0.035 mg/kg and 0.17 mg/kg NLX) aligned with W. Schiffer Rat Brain atlas using PMOD . NLX was given 15 min after \[^{11}\text{C}]\text{CFN administration to assess displacement. The top row corresponds to the average images obtained at 0-15 min and the bottom row corresponds to averaged images obtained at 15-30 min, in which at 15 min, NLX was injected for 0.035 and 0.17 mg/kg doses.}
Figure 4

MOR occupancy profiles after IV NLX for two doses (circle, 0.035 mg/kg; rectangle, 0.17 mg/kg). Occupancy data averaged for each time point were plotted with a sigmoidal function. NLX 0.035 mg/kg corresponds to 0.4 mg HED and NLX 0.17 mg/kg to 2 mg HED. Error bars correspond to standard deviations.
Figure 5

Averaged plasma concentration after IV NLX in rats. NLX was administered intravenously for each of the two doses (0.035 mg/kg, n=3; 0.17 mg/kg, n=3).

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