NYX-2925, an NMDA receptor modulator with glycine site partial agonist-like properties, induces rapid and long-lasting analgesic effect in multiple rat models of neuropathic pain

Poster # 221

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ABSTRACT

Aim of Investigation: NYX-2925 is an orally bioavailable small molecule NMDA receptor modulator. The present studies examine the effect of NYX-2925 in multiple rat models of neuropathic pain.

Methods: The analgesic effects of NYX-2925 were evaluated in the rat Bennett model (chronic sciatic nerve constriction), the Taxol model of chemotherapy-induced pain, the streptozotocin (STZ) model of diabetic neuropathy, the formalin model of persistent pain, and the tail flick model of acute pain. Potential sedative / ataxic effects were also examined in the rota-rod test.

Results: A single oral dose of NYX-2925 produced rapid-acting (1 h post-dosing) and long-lasting (24 h and 1 week post-dosing) mechanical and thermal analgesia in the Bennett model (mechanical EC_{50} = 0.3 mg/kg PO; thermal EC_{50} = 0.4 mg/kg PO), and mechanical analgesia in the STZ model (EC_{50} = 0.3 mg/kg PO) and Taxol (EC_{50} = 5 mg/kg PO) models. In contrast, the gabapentin (150 mg/kg PO) positive control was not analgesic in the Taxol model, and only produced an analgesic effect 1 h but not 24 h or 1 week post-dosing in the Bennett and STZ models. NYX-2925 also reduced flinching in the late phase of the formalin test 1 h post dosing (EC_{50} = 0.2 mg/kg PO) to a similar degree as gabapentin (150 mg/kg PO). NYX-2925 (1 mg/kg PO) was ineffective the tail flick model of acute pain. Lastly, NYX-2925 (1-100 mg/kg PO) did not induce a sedative / ataxic effect in the Rota-rod test when measured up to 2 h post-dosing, whereas a therapeutic dose of gabapentin (150 mg/kg PO) did produce sedative / ataxic effects at 1 h and 2 h post-dosing.

Conclusions: These data show that NYX-2925 has therapeutic potential as a rapid acting and long-lasting therapeutic for the treatment of a variety of neuropathic pain conditions with no sedative or ataxic effects.

Note: see poster # 223 for additional in vitro and in vivo characteristics of NYX-2925

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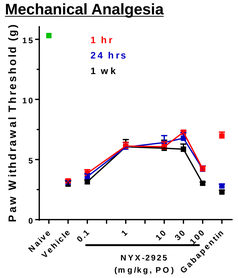
FINANCIAL DISCLOSURES

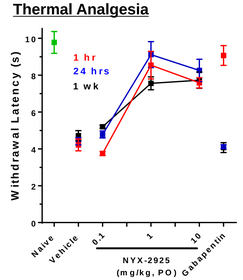
NH, EC, AG, JP, JD, TM, MK, RK, CC and JM are employees of Aptinyx Inc. JB is a consultant for Aptinyx Inc.

1 NYX-2925 Produces Long-lasting Mechanical Analgesia in the Bennett / CCI model and Thermal Analgesia in the SNI Model of Neuropathic Pain

Mechanical Analgesia



Thermal Analgesia



% Analgesia	1 h	24 h	1 wk
NYX-2925 0.1 mg/kg	10.2 ± 4.5	9.0 ± 3.7	6.2 ± 3.8
NYX-2925 1 mg/kg	40.0 ± 3.2	39.3 ± 4	41.1 ± 6.6
NYX-2925 10 mg/kg	38.5 ± 4.1	41.34 ± 5.5	41.2 ± 7.1
NYX-2925 30 mg/kg	53.1 ± 1.1	49.4 ± 4.2	43.5 ± 4.3
NYX-2925 100 mg/kg	15.1 ± 1.8	18.9 ± 3.7	4.7 ± 1.2
Gabapentin 150 mg/kg	48.7 ± 3.0	-6.5 ± 3.9	-12.0 ± 3.9
Vehicle	0 ± 2.0	0 ± 3.1	0 ± 5.1

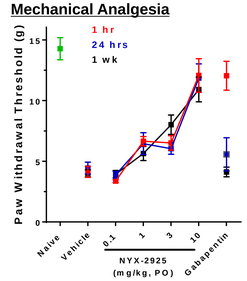
% Analgesia	1 h	24 h	1 wk
NYX-2925 0.1 mg/kg	-9.3 ± 1.9	7.5 ± 3.2	9.6 ± 1.3
NYX-2925 1 mg/kg	86.3 ± 12.9	113.6 ± 18.9	59.9 ± 5.9
NYX-2925 10 mg/kg	67.2 ± 4.6	78 ± 10.6	59.3 ± 7.5
Gabapentin 150 mg/kg	96.8 ± 9.1	-6 ± 2	-12.7 ± 4.5
Vehicle	0 ± 5.5	0 ± 3.4	0 ± 4.7

Thermal Hyperalgesia:
%Analgesia = [(treated ipsilateral latency – mean of vehicle ipsilateral latency) / (mean of vehicle contralateral latency – mean of vehicle ipsilateral latency)] × 100.

For the mechanical analgesia, rats were anesthetized using inhaled isoflurane (2.5%). Chronic constriction injury surgery was performed as previously described (Bennett and Xie, 1988). The sciatic nerve was loosely ligated and the ligatures were tightened to keep the suture in place. For the thermal analgesia studies, a spared nerve injury surgery was performed and the common peroneal and tibial branches of the sciatic nerve were ligated and cut. The sural branch was spared (Decosterd and Woolf, 2000). Testing for both tests occurred 1-2 weeks post-surgery. Testing occurred 1 h, 24 h and 1 wk post-dosing with NYX-2925 (0.1-100 mg/kg PO), gabapentin (150 mg/kg PO), or vehicle (0.5% Na-CMC in 0.9% sterile saline). Mechanical analgesia was measured using Von Frey filaments and Dixon's up-down method (Chaplan et al., 1994). Von Frey filaments were pressed perpendicularly to the plantar surface of the affected (ipsilateral) hindpaw. Filaments were held for 6 s. If an obvious hind paw withdrawal was not observed, the next larger filament was used in the same manner. In case of a response, a lower filament was used. This was repeated until six responses were collected. Thermal analgesia was measured using the Plantar Test (Hargreaves Apparatus; Ugo Basile, Italy). Rats were placed in a Plexiglas compartment above the glass surface of the instrument. The infrared source placed under the glass floor was positioned directly beneath the hind paw. Rats received 3 withdrawal measurements for both the non-affected (contralateral) paw and affected (ipsilateral) paw, with a 5 min interval between trials.

2 NYX-2925 Produces Long-Lasting Mechanical Analgesia in the STZ Model of Diabetic Peripheral Neuropathy

Mechanical Analgesia



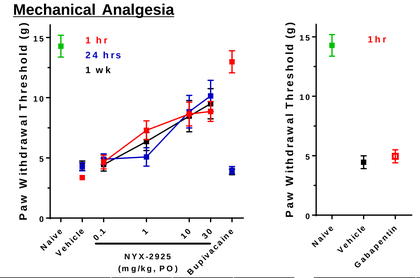
% Analgesia	1 h	24 h	1 wk
NYX-2925 0.1 mg/kg	-13.2 ± 2.6	-8.1 ± 5.6	-0.8 ± 5.4
NYX-2925 1 mg/kg	37.7 ± 4.4	28.0 ± 10.5	25.1 ± 7.7
NYX-2925 3 mg/kg	34.2 ± 6.7	25.5 ± 7.2	51.8 ± 7.3
NYX-2925 10 mg/kg	80.6 ± 10.0	79.4 ± 7.8	74.7 ± 6.7
Gabapentin 150 mg/kg	81.5 ± 7.9	10.9 ± 17.6	1.9 ± 7.1
Vehicle	0 ± 8.9	0 ± 8.8	0 ± 5.3

Mechanical Hypersensitivity:
%Analgesia = [(poststrug threshold - post-injured baseline threshold) / (15g - post-injured baseline threshold)] × 100.

Rats were injected with streptozotocin (STZ; Sigma S0130) at 20 mg/kg ip daily for 5 consecutive days after which they were given drinking water supplemented with sucrose (15 g/L) for 48 h to limit early mortality. Blood glucose levels were assessed weekly to evaluate hyperglycemia and the rats with fasting blood glucose of over 15 mmol/L (280 mg/dL) were selected to be included in the studies of diabetic neuropathy. Animals were tested 3 month post-STZ. Testing occurred 1 h, 24 h and 1 wk post-dosing with NYX-2925 (0.1-10 mg/kg PO), gabapentin (150 mg/kg PO), or 0.5% Na-CMC in 0.9% sterile saline. Mechanical analgesia was measured using Von Frey filaments and Dixon's up-down method (Chaplan et al., 1994).

3 NYX-2925 Produces a Long Lasting Mechanical Analgesic Effect in the Taxol® Model of Neuropathic Pain

Mechanical Analgesia



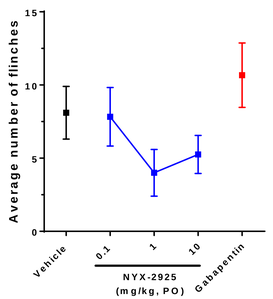
% Analgesia	1 h	24 h	1 wk
NYX-2925 0.1 mg/kg	19.3 ± 8.4	11.27 ± 7	2.2 ± 9.4
NYX-2925 1 mg/kg	47.9 ± 7.7	8.6 ± 10.8	28.0 ± 9.1
NYX-2925 10 mg/kg	56.7 ± 9.6	46.0 ± 14.0	44.4 ±12.8
NYX-2925 30 mg/kg	61.8 ± 6.2	43.1 ± 9.8	58.4 ± 12.5
Bupivacaine 150 µg/µL	89.5 ± 5.8	-5.5 ± 5.3	-7.6 ± 4.6
Vehicle	0 ± 1.8	0 ± 5.4	0 ± 7.0

% Analgesia	1 h
Gabapentin 150 mg/kg	0
Vehicle	8.7

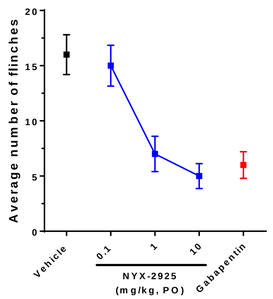
To induce neuropathy, rats were dosed with Taxol® (2 mg/kg, IP) or vehicle (0.5% Na-CMC in 0.9% sterile saline, 1 ml/kg) on D1, D3, D5, and D7 (Polomano et al. 2001). Testing was conducted 1 month after the start of Taxol® dosing. Testing occurred 1 h, 24 h and 1 wk post-dosing with NYX-2925 (0.1-30 mg/kg PO), gabapentin (150 mg/kg PO), or 0.5% Na-CMC in 0.9% sterile saline. Bupivacaine (150 µg in 50 µl SC into the footpad) was administered 30 min before testing. Mechanical analgesia was measured using Von Frey filaments and Dixon's up-down method (Chaplan et al., 1994).

4 NYX-2925 Reduces Spontaneous Flinching Behavior in Rats Administered Formalin in Late Phase Post-Formalin Injection

Early Phase (0-10 min post formalin)

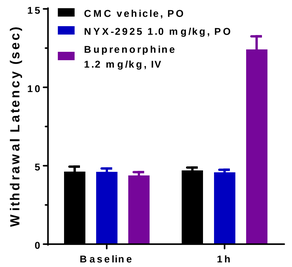


Late Phase (30-55 min post formalin)



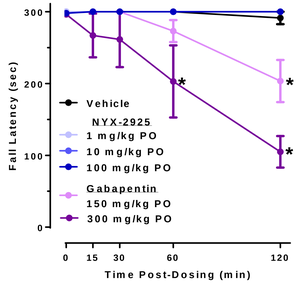
The formalin test of neuropathic pain was conducted as previously described (Wood et al., 2008). Rats were dosed with vehicle (0.5% CMC), NYX-2925 (0.1, 1, or 10 mg/kg), or the positive control PO 30 min prior to the formalin injections. In order for the rats to acclimate, they were placed individually on the formalin rack 15 min prior to the formalin injections. For better visualization, the rack was equipped with a mirror-attached underneath. At T=0, each rat was restrained and 50 ul of 5% formalin (in injectable saline) was injected into its dorsal center of its right paw using 0.5 ml insulin syringe. Recording was started immediately after injections and continued for 65 min. Scoring of the data was performed in a blinded manner. For the pain scores number of flinches and licking/biting were counted. NYX-2925 showed similar analgesic effects for the late phase licking/biting as that of the flinches.

5 NYX-2925 Does Not Show an Analgesic Effect in the Tail Flick Model of Acute Pain



Each rat was restrained and placed on the tail flick apparatus (Ugo Basile Tail Flic Cat#: 37360) such that the tip of the tail (at 2-2.5 cm) was placed on top of the mounted window above the IR heat source. IR frequency was set at 50 and the cut off time at 15 sec. The baseline latency (sec) at which the rat removed its tail from the heat source was measured 15 minutes prior to dosing. Rats were then dosed with NYX-2925 (1 mg/kg, PO), vehicle (0.5% Na-CMC, PO) or the positive control (buprenorphine 1.2 mg/kg, IV). Withdrawal latencies were measures at 1 h post dosing. Each rat's measurement was taken 2 to 3 times (5 min apart) and averaged.

6 NYX-2925 Does Not Show Sedative/Ataxic Side Effects in the Rat Rota-Rod Test



Animals were habituated 3 times before the start of testing with a fourth habituation session occurring right before dosing (0 min timepoint). A fixed speed version (16 rpm) of the Rota-rod test was used. Adult male Sprague Dawley rats were dosed with gabapentin (150,300 mg/kg PO), NYX-2925 (1-100 mg/kg PO) or 0.5% Na-CMC in 0.9% saline vehicle (1 ml/kg PO) 15 min before the first test session. Animals were re-tested 30, 60, 120 min post-dosing. Mean (± SEM) latency to fall of the Rota-Rod was recorded. N = 6-11 per group. * P < .05 Fisher's PLSD post hoc test gabapentin (150 or 300 mg/kg) vs. vehicle for each timepoint.