The dopamine β-hydroxylase inhibitor, nepicastat, suppresses different chocolate-motivated behaviours in rats

Alessandro Zaru

Neuroscience Institute, Section of Cagliari, National Research Council of Italy

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INTRODUCTION

- Recent lines of experimental evidence suggest that pharmacological inhibition of dopamine β-hydroxylase (DBH), the cathecolamine biosynthetic enzyme that converts dopamine (DA) to norepinephrine (NA), may attenuate reinstatement of cocaine-seeking in rats (Neuropsychopharmacology 35:2440-2449, 2011).
- Chocolate is a natural reward capable of driving, in rodents and humans, multiple behaviours that resemble those motivated by drugs of abuse. For instance, rats steadily maintain exceptionally high levels of operant responding (e.g., lever-pressing) to access chocolate-containing preparations, similarly to the behaviour performed for cocaine.
- The present study was designed to investigate whether DBH inhibition could affect different behaviours motivated by a chocolate-flavoured beverage in rats. To this end, nepicastat – a direct, competitive inhibitor of DBH – was tested in rats trained to lever-respond for a chocolate-flavoured beverage.
- Three different experiments were performed:
  - ***Experiment 1*** evaluated the effect of treatment with nepicastat on self-administration of the chocolate-flavoured beverage in rats exposed to the Fixed Ratio (FR) schedule of reinforcement, in which the response requirement (RR; i.e., the "cost" of each chocolate presentation in terms of number of responses on the lever) was predetermined and kept fixed throughout the session; this schedule of reinforcement provided a measure of the reinforcing properties of chocolate;
  - ***Experiment 2*** evaluated the effect of treatment with nepicastat on self-administration of the chocolate-flavoured beverage in rats exposed to the Progressive Ratio (PR) schedule of reinforcement, in which RR was progressively increased after the delivery of each chocolate presentation; the lowest ratio not completed (named breakpoint) was taken as measure of the motivational properties of chocolate;
  - ***Experiment 3*** evaluated the effect of treatment with nepicastat on reinstatement of chocolate-seeking behaviour in rats in which operant responding for the chocolate-flavoured beverage had been extinguished making the chocolate-flavoured beverage unavailable; the subsequent presentation of a complex of stimuli previously associated to availability of the chocolate-flavoured beverage reinstated lever-responding behaviour, modeling relapse episodes and loss of control over chocolate.
  - ***Experiment 4*** evaluated the effect of treatment with nepicastat on self-administration of regular food pellets in rats exposed to the FR schedule of reinforcement. This experiment was performed to clarify whether nepicastat effect was limited to the reinforcing properties of a highly palatable food, like the chocolate-flavoured beverage, or was extended to the reinforcing properties of regular food elicited by appetite.

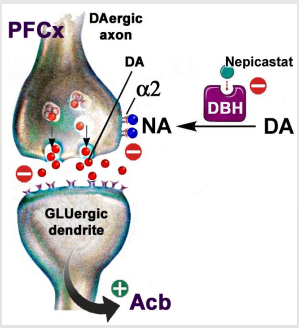
  An additional experiment (***Experiment 5***) evaluated the effect of nepicastat on spontaneous locomotor activity in rats.

MATERIALS AND METHODS

- **Animals:** Male, adult Wistar rats (*n*=12).
- **Chocolate-flavoured beverage:** 5% (w/v) Nesquik® in water.
- **Treatment groups:** 0, 25, 50, and 100 mg/kg nepicastat (Latin-square design).
- **Nepicastat preparation & route of administration:** Dissolved in saline with 0.3% DMSO and 0.3% Tween 80; administered intraperitoneally (injection volume: 2 ml/kg).
- **Experimental procedures:**
  - **Experiment 1 -** Operant, oral self-administration of the chocolate-flavoured beverage in daily 30-min sessions under the FR10 schedule of reinforcement; regular food pellets and water always available in the homecage.
  - **Experiment 2** - Operant, oral chocolate self-administration in daily 30-min sessions under (a) FR10 (training sessions) and (b) PR (10, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, etc.) (testing sessions) schedules of reinforcement; regular food pellets and water always available in the homecage.
  - **Experiment 3** - Operant, oral self-administration of the chocolate-flavoured beverage in 15 daily 30-min sessions under the FR10 schedule of reinforcement (maintenance phase); subsequently, 4-7 daily sessions of extinction responding (extinction phase) during which the chocolate-flavoured beverage was unavailable and lever-responding unreinforced; in the reinstatement session, a stimulus complex previously associated to availability of the chocolate-flavoured beverage was presented; regular food pellets and water always available in the homecage.
  - **Experiment 4** - Operant, oral self-administration of regular food micropellets (45 mg) in daily 30-min sessions under the FR10 schedule of reinforcement; rats underwent a mild deprivation regimen by limiting the amount of regular food pellets in the homecage; water always available.
  - **Experiment 5** - Single exposure to a 30-min session of spontaneous locomotor activity in computer-operated, photocell-equipped, Plexiglas test cages; two different sessions occurring 3 and 24 hours after nepicastat administration (independent groups of rats were used in each session).

CONCLUSIONS

- Administration of the DBH inhibitor, nepicastat, dose-dependently suppressed the reinforcing (Experiment 1) and motivational (Experiment 2) properties of a chocolate-flavoured beverage in rats.
- Administration of nepicastat also abolished reinstatement of seeking behaviour for the chocolate-flavoured beverage (Experiment 3).
- The suppressant effect of nepicastat was not specific for the operant responding driven by the hedonic qualities of food; indeed, nepicastat was equally effective in inhibiting operant responding maintained by appetite in rats subjected to a food-restriction regimen calibrated to generate lever-responding for regular food pellets (Experiment 4) equal to that produced by the chocolate-flavoured beverage in fed rats.
- The results of the ancillary, locomotor study (Experiment 5) suggest that nepicastat-induced blockade of food-motivated behaviours can not be attributed to nepicastat-induced sedation, inability to perform the operant task, or malaise.
- **These results suggest that nepicastat may be effective in reducing the reinforcing properties of food, when sustained by either palatability or appetite; these results also suggest that a common neural substrate likely controls both conditions.**
- **Possible mechanism of action:** the ability of nepicastat to suppress the reinstatement of cocaine seeking has been attributed to the reduced NA production and the consequent loss of an α1-adrenoceptor-mediated stimulatory tonus on mesolimbic DArgic neurons, which is needed so that environmental stimuli are able to trigger DA release in the nucleus accumbens. However, we recently found that nepicastat not only reduced – as expected by DBH inhibition – NA release in different brain areas, but also caused a marked increase in DA release in the prefrontal cortex (PFCx).

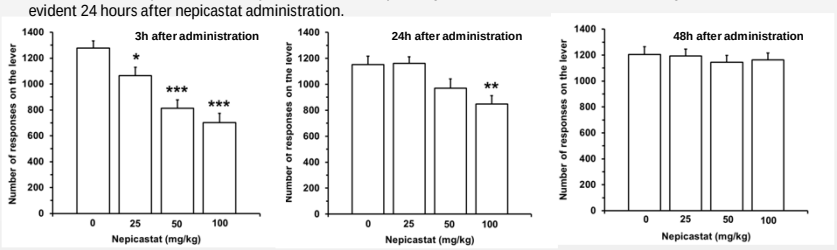


To explain this effect, we postulated that nepicastat, by removing NA from α2-adrenoceptors, would relieve NAergic and DAergic terminals in the PFCx from the inhibitory control exerted by NA, thereby causing an unrestrained DA release from these terminals. Since cortical DA is thought to exert an inhibitory control on GLUergic excitatory projections from the PFCx to the nucleus accumbens (Acb), which play a critical role in relapse to drug and food seeking, we suggest that DA accumulation in the PFCx may contribute to the suppressant effect of nepicastat on the reinstatement of cocaine and food seeking.

The author declare no conflict of interest

Experiment 1 — Reducing effect of nepicastat on operant chocolate self-administration in rats under the FR schedule of reinforcement (index of the reinforcing properties of chocolate)

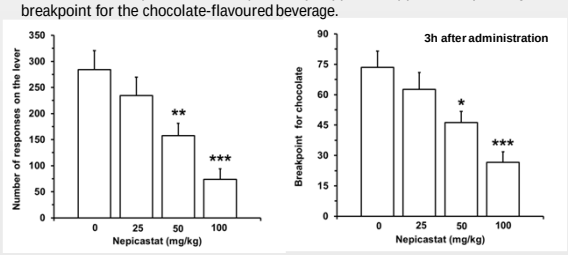
Treatment with nepicastat markedly reduced lever-responding for the chocolate-flavoured beverage. This effect was still evident 24 hours after nepicastat administration.



Each bar is the mean ± SEM of n=12 rats. ★: P<0.05, ★★: P<0.01, and ★★★: P<0.001 with respect to vehicle-treated rats (Newman-Keuls test).

Experiment 2 — Suppressing effect of nepicastat on operant chocolate self-administration in rats under the PR schedule of reinforcement (index of the motivational properties of chocolate)

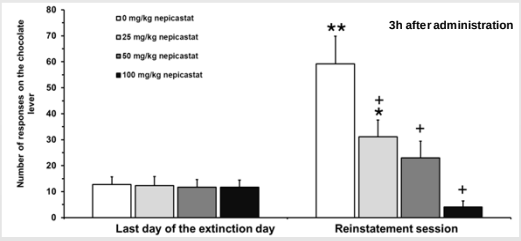
Treatment with nepicastat dose-dependently suppressed (a) lever-responding for the chocolate-flavoured beverage and (b) breakpoint for the chocolate-flavoured beverage.



Each bar is the mean ± SEM of n=9 rats. ★: P<0.05, ★★: P<0.01, and ★★★: P<0.0005 with respect to vehicle-treated rats (Newman-Keuls test).

Experiment 3 — Suppressing effect of nepicastat on reinstatement of chocolate-seeking behaviour in rats (model of relapse episodes and loss of control over chocolate)

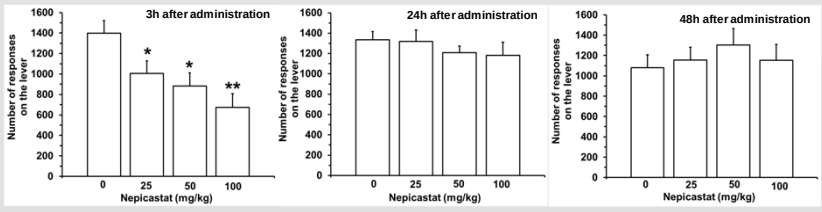
Under the vehicle condition, presentation of the stimulus complex previously associated to the chocolate-flavoured beverage robustly reinstated lever-responding. Treatment with nepicastat completely abolished lever-responding. One nepicastat-treated rat totally avoided responding.



Each bar is the mean ± SEM of n=8 rats. ★ P<0.05 and ★★: P<0.0005 with respect to the same rat group in the last session of the extinction phase (Newman-Keuls test); +: P<0.0005 with respect to the vehicle-treated rat group in the reinstatement session (Newman-Keuls test).

Experiment 4 — Reducing effect of nepicastat on operant self-administration of regular food pellets in rats under the FR schedule of reinforcement (experiment control)

Treatment with nepicastat markedly reduced also lever-responding for food pellets. These results indicate that the suppressant effect of nepicastat was not specific for the operant responding driven by the hedonic qualities of food.



Each bar is the mean ± SEM of n=12 rats. ★: P<0.05 and ★★: P<0.01 with respect to vehicle-treated rats (Newman-Keuls test).

Experiment 5 — None of the nepicastat doses that reduced chocolate-motivated behaviours affected, even minimally, spontaneous locomotor activity (experiment control)

	Nepicastat (mg/kg)			
	0	25	50	100
Session 1 (3 hours after nepicastat administration)	10583 ± 697	11145 ± 1106	9158 ± 605	9029 ± 838
Session 2 (24 hours after nepicastat administration)	13376 ± 617	14149 ± 908	13754 ± 1609	12294 ± 1121

The measured variable was the total number of counts (photocell breaks) recorded automatically by the apparatus in each session. Each value represents the mean ± SEM of n=8-9 rats.