The effects of clonidine and yohimbine in the tail flick and hot plate tests in the naked mole rat (*Heterocephalus glaber*)

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Research note

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Abstract

Objective: The naked mole rat (NMR) (*Heterocephalus glaber)* is increasingly considered an important biomedical research model for various conditions like hypoxic brain injury, cancer and nociception. This study was designed to investigate the effects of clonidine and yohimbine, an alpha-2 (α_2) adrenoceptor agonist and antagonist respectively in the tail flick and hot plate tests.

Results: A significant difference in tail flick latency was noted between saline control and 30µg/kg clonidine, which was reduced after administration of 30µg/kg yohimbine. A significant difference in hot plate latency was also noted between saline control and 30µg/kg clodinine during the periods 30, 45, 60, 75 and 90 minutes after administration, and between saline control and 10µg/kg clonidine during 30 minutes after administration. The hot plate latency by 30µg/kg clonidine was also reduced by 30µg/kg yohimbine during 30 minutes after administration. Since the tail-flick and hot plate tests mediate the effects at spinal and supraspinal levels respectively, the present study indicates the presence and involvement of noradrenergic receptors in thermal antinociception at spinal and supraspinal levels of the NMR, similar to what has been found in other mammals.

Introduction

NMRs are insensitive to some types of pain (1–3). Hence their bio prospecting as research animal models for nociception with the aim to further understand mechanisms involved in various biological diseases affecting humans (1, 2, 4).

The involvement of opioid (5, 6), cholinergic (8, 9) and GABA (11, 12) receptor pathways in NMR pain mechanisms has been reported. Noradrenergic receptor system involvement in nociception is reported in other animals such as; rats (13), pigs (14), dog and cats (15), Speke's hinged tortoise (16), small ruminants (17), and frog (18). To our knowledge, the involvement of the noradrenergic receptor system in antinociception against thermal stimuli has not been previously studied in the NMR hence the basis of this study.

Materials And Methods

Animals, husbandry and study design

A total of 128 NMRs captured in Makueni County, Kenya, were kept at a controlled environment at South Eastern Kenya University. Colonies were housed in Perspex cages with wood shavings as bedding material.

After one month acclimatization, NMRs weighing between 25–35 grams in groups of mixed sex per drug or control dose were used in the study with each animal acting as experimental unit. Females and males were equally distributed in the groups. Each NMR was randomly selected and used only once. The

experimenter was blinded and thus not aware of the drugs or vehicle injected until after data analysis. The sample size (n) was based on a power analysis resulting in a group size (n) of eight animals.

Tests were performed similar to (8) using tail flick and hot plate analgesiometers (H.L Scientific, Ambala, India). Tests were carried out 30 minutes after receptor ligands or control intraperitoneal injection. Latency periods were then recorded in seconds with cutoff points at 10 and 30 seconds for tail flick and hot plate respectively to avoid injury. Each animals was euthanized by cervical dislocation after the experiment.

Drugs

Clonidine hydrochloride (CL) and yohimbine hydrochloride (Yoh) both from Sigma-Aldrich (Taufkirchen, Germany) and 0.9 % normal saline (NS).

Statistical analysis

Data were analyzed in Graph Pad Prism version 5.0. $P < 0.05$ was considered a statistically significant difference.

Results

Doses used in this study were based on studies by (19). The effect of 1–30 µg/kg CL and NS control on tail flick latency is shown in Fig. 1a. The effect of 1–30 µg/kg CL and NS control on the hot plate latency is shown in Fig. 2a. The effects of co-administration of Yoh with CL in both the tail flick and hot plate tests is shown in Figs. 1b and 2b respectively.

Discussion

Noradrenergic receptors play an inhibitory role at pre and post synaptic neurons and occur at both central (CNS) and peripheral nervous systems (PNS) where they function as G protein-coupled receptors (20, 21). They are divided into α1 and α2 types (22, 23).

Activation of α2 adrenoceptors by their agonists like CL causes sedation, muscle relaxation and analgesia (24, 25). CL has a high affinity for α2 adrenoceptors at both CNS and PNS with a predilection of 200:1 for α2 compared to α1 adrenoceptors (24). Yoh is a prototypical α2 adrenergic receptor antagonist due to its high selectivity (26). To study the spinal nociceptive reflexes, we performed the tail flick test where noxious thermal stimuli from radiant heat is applied on the tail (27). The significant difference in tail flick when 30µg/kg CL latency period was compared to that of NS and the reversal of the effects by Yoh indicates the presence and involvement of noradrenergic receptors at spinal level in the NMR. The increase in tail flick latency following administration of CL and the reversal of the effects by Yoh is also reported in rats (28) and mice (29, 30).

To study supra spinal effects, we performed the hot plate test where thermal noxious stimuli from a hot plate heated at 60 ± 2 ºC was applied (7, 8). Significant difference in hot plate latency when 30µg/kg CL was compared to that of NS and the reversal of the effects by Yoh indicates the presence and involvement of noradrenergic receptors at supra spinal level in the NMR. This is also reported in rats (31) and mice (29).

In conclusion, this study demonstrates involvement of noradrenergic receptor system in thermal antinociception of NMR at both CNS and PNS levels, similar to what has been reported in other rodents such as rats and mice. An indication that NMRs can serve as animal models for studying pain mechanisms involving the noradrenergic receptor system.

Limitations

Wild type NMR of unknown ages were used. Their genetic profile was likely diverse, giving the experiments a lower precision. The ligands used may also have affected other receptor systems, hence the effects noted may not be purely due to noradrenergic receptors involvement.

Abbreviations

NMR; Naked mole rat: GABA; g-amino butyric acid: α; alpha: kg; kilograms: µg; micro grams: CNS; central nervous system: PNS; peripheral nervous system: NACL; sodium chloride

Declarations

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Author contributions

RM conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

TK conceived and designed the experiments; contributed reagents, materials, analysis tools or data, reviewed and approved the paper. KA conceived and designed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools, reviewed and approved the paper.

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Availability of data and materials

All the data and results generated and analyzed have been presented in this published article

Ethical approval and consent to participate

The experiments were conducted after research approval licensing (Ref; KWS/BRM/5001) and obtaining a naked mole rat capture permit (Ref; KWS/904) from Kenya Wildlife Service (KWS). The study also adhered to prevention of cruelty to animals act, chapter 360, laws of Kenya and where applicable to Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The study was also approved by the biosafety, animal use and ethics committee of the Faculty of Veterinary Medicine, University of Nairobi (Ref; FVM BAUEC/2020/275).

Consent for publication

Not applicable.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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Figures

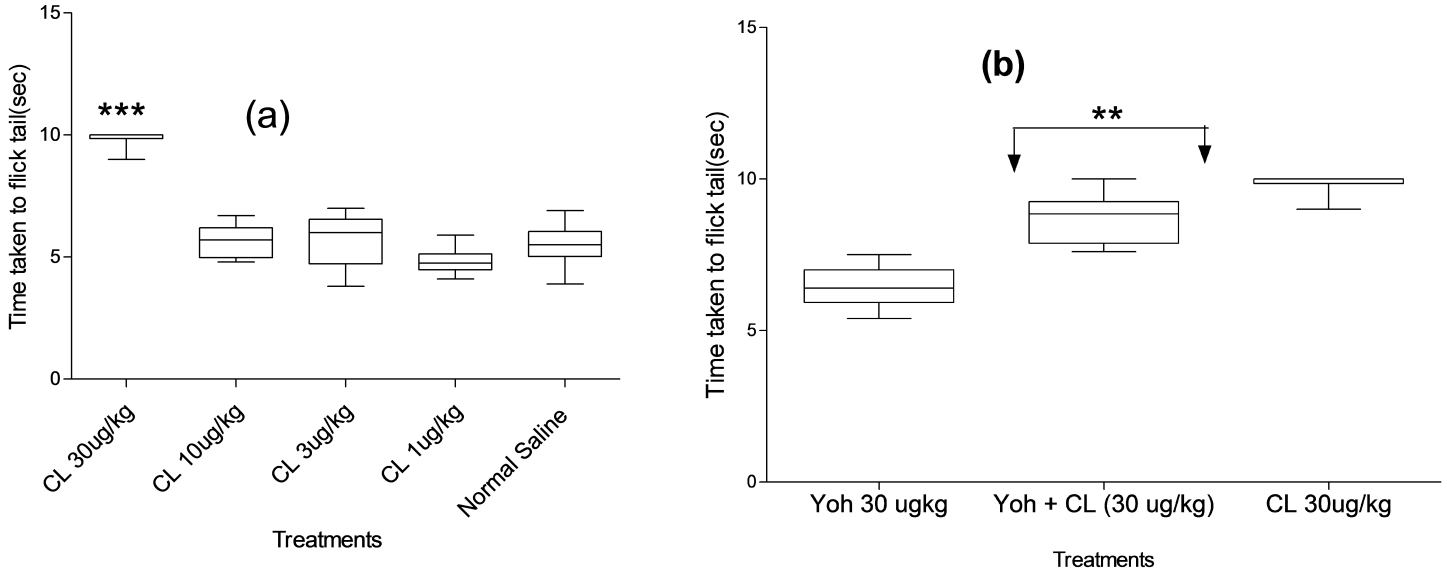

Figure 1

(a) The effects of CL at various doses (treatment) and NS (control). Significant differences ($p<0.0001$***) in tail flick latency were found between 30µg/kg CL compared to NS and CL dosages 1, 3 & 10µg/kg. (b) Latency period when CL was co administered with Yoh significantly differed ($p<0.05$**) with the effects of 30 µg/kg CL alone. The whiskers show minimum and maximum latency periods while the horizontal bar within the boxes show median latency in seconds. Number of animals (n) = 8 for all doses. Data were analyzed using one way ANOVA with Tukey's Multiple Comparison Test.

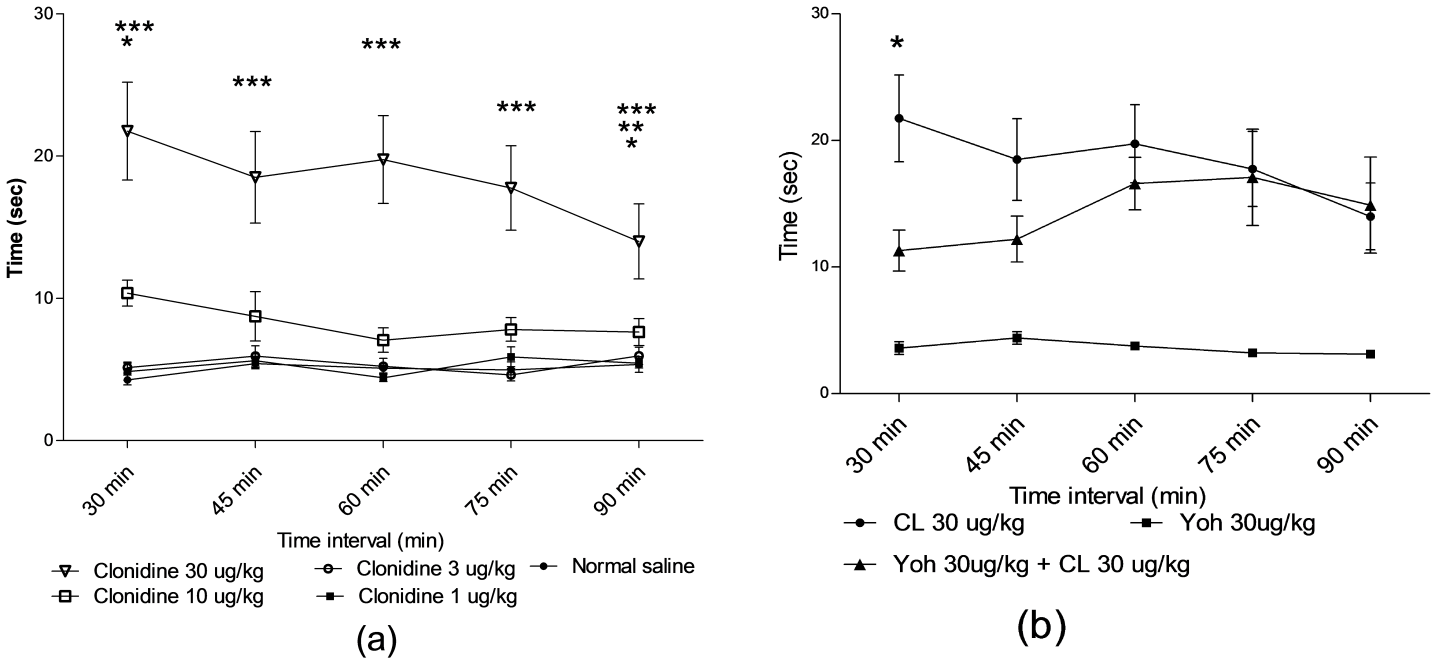

Figure 2

(a) Effect of NS (control) and CL (treatment) at various doses on hot plate latency. There were significant differences when; 30µg/kg CL was compared to NS control and 1, 3, 10 µg/kg CL (P<0.001***) at 30, 45, 60 and 75 minutes; NS and 10µg/kg CL (P < 0.05*) when compared at 30 minutes; 30µg/kg CL when compared to 10 µg/kg CL (P < 0.05*) , 3 µg/kg CL (P<0.01**) & NS (P<0.001***) at 90 minutes (b) Latency period when Yoh was co administered with CL significantly differed with that of CL administered alone at 30 minutes (P < 0.05*). Data are shown as mean time (±SEM) in seconds. Number of animals (n) = 8 for all doses. Data were analyzed using Two way repeated measures ANOVA with Bonferroni posttests.

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