Capsaicin Instillation in the eye: an experimental model of the study of nociception in chicken (*Gallus gallus domesticus*)

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Abstract

Ethogram and effective capsaicin concentration established in five months old indigenous chicken. In the ethogram development, capsaicin concentration ranging from 10^{-2} g/ml to 10^{-9}g/ml instilled in the eye of indigenous chicken induced pain related behaviors. The consistent pain related behaviors induced due to capsaicin instillation chosen and counted. Capsaicin diluent and normal saline were used as controls. The most effective capsaicin concentration was determined by instilling capsaicin concentrations 10^{-2} - 10^{-9}g/ml in the eye of indigenous chicken in blocks of five minutes in an hour. The time spent in painful behaviors was recorded using a stopwatch. The concentration which induced response immediately after instillation was chosen. Capsaicin diluent and normal saline were used as controls. In ethogram development, the painful behaviors chosen were head shaking, eye scratching, blinking, eye closure, and partial eye closure. The collected data was analyzed using graph prism 5.0 and means separated using Tukey's application. The most effective capsaicin dose determined from capsaicin concentrations 10^{-2} - 10^{-9}g/ml was 10^{-2} g/ml. A model for testing pain behavior related in chicken in chicken is developed in these experiments. The study indicates that chicken display different behaviors when in pain though they are highly stoic. Capsaicin diluent and normal saline used as controls can be used independently for related experiments because they yielded same results.. Capsaicin-induced pain can be used in studying nociceptive mechanisms in chicken as evidenced from this study and other related studies such those done by Kanui (1990) in crocodiles.

Key words Capsaicin, response, ethogram, TRPV I, nociception

1.0 Introduction

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), is a phenolic compound found in chili peppers and produces burning sensation, irritation, and pain on contact. Paradoxically, it can also be used to manage pain when applied on correct unpleasant sensations are not felt by birds (Fattori *et al.,* 2016). Capsaicin dose and frequency. However, these is well absorbed when administered either topically or orally (Suresh *et al.,* 2010). Capsaicin produces pain which makes it an important tool (Fattori *et al.,* 2016). Capsaicin has a nonpolar phenolic structure and cannot be solubilized in water. The main solvents used to extract and maintain capsaicin properties are nonpolar solvents which include ether, benzene, dimethyl sulfoxide, and acetone. Ethanol (ethyl alcohol) can be used as a solvent due to its mixed properties. Cloning transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor gave light on the mechanism by which capsaicin induces pain. The administration of capsaicin in animals elucidate the function of TRPV1 and aid the knowledge of pain processing and modulation. The discovery of TRPV1 is essential to validate capsaicin-induced pain models (Fattori *et al.,* 2016). Capsaicin can be administered by a number of different routes. In humans, it can be administered by exposure to the ingredient through consumption and in self-defending actions (pepper sprays), and as topical analgesics. For instance, capsaicin was used topically in modality-specific facilitation of non-injurious sharp mechanical pain (Shabes *et al.,* 2021).

Capsaicin administration methods in basic science studies include intradermal and or intra-plantar injections. In reptiles, capsaicin was used in the eye of crocodiles (Kanui *et al*., 1990). Nociception is the encoding of a noxious stimulus *i.e.*, an actual or potential tissue-damaging event, and its transduction into electric signals. Noxious stimuli are detected by nerve endings found throughout the body and originating from the parasympathetic nervous system (PSNs), which represent the first element of a poly-neuronal chain leading to the perception of pain (Frias and Merighi 2016; Kanui *et al*., 1987). The nociceptors, (PSNs) respond to nociceptive stimuli which under certain conditions can be activated by innocuous forms of the same stimulus like heat and cold nociceptors. Nociceptors are polymodal receptors because they respond to stimuli of a heterogeneous nature: mechanical (e.g., high pressure), thermal (too high or too low temperatures), and chemical (Frias and Merighi, 2016). Activated nociceptors in skin, muscles, joints, or viscera generate a nerve signal (action potential) that is transferred to the somatosensory cortex, the parieto-insular cortex, and to the anterior cingulate cortex where the sensation of pain is perceived. In chicken there is no adequate information on whether capsaicin can cause pain in the eye of chicken or not. Nor is there information on the effective capsaicin effective dose if at all it causes pain when ocularly applied. The study demonstrates the effects of capsaicin in the eye by developing an ethogram and establishing the effective dose of capsaicin in the eye of chicken (*Gallus gallus domesticus*).

2.0 Methods

2.1 Drugs

The capsaicin was sourced from Sigma Aldrich company, U.S.A., absolute ethanol (ethyl alcohol) was got from PAC L.P.A. Roper company, tween 20 (polysorbate 20) was from Twiga Chemical Industries Ltd., while normal saline was acquired from Crown Healthcare company. Capsaicin was dissolved in ethanol (ethyl alcohol) and stabilized in tween 20 (polyoxyethylene sorbitol ester) and diluted in normal saline obtained from Sigma Aldrich (Gamse *et al.*, 1982; Kanui *et al.*, 1990). Capsaicin vehicle diluent was made by mixing ethanol 10% (ethyl alcohol), tween 20 10%, and normal saline 80%. A stock solution of 10^{-2}g/ml was made by having capsaicin 1g weighed electronically and dissolved in 10 ml absolute ethanol 10% and Tween 20 10% and of 10 ml were measured using a 10 ml measuring cylinder. Mixing was thoroughly done through shaking in a volumetric bottle. Normal saline 80% of 80 ml was added. A thorough shaking was followed for proper dissolution. The resulting solution was 10^{-2}g/ml capsaicin, stock solution. Subsequent capsaicin concentrations (10^{-3} - 10^{-9}g/ml) were obtained by diluting the stock solution with normal saline (Kanui *et al*., 1990).

2.2 The experimental birds

Experimental birds, aged four months of both sexes were purchased from a local hatchery in Mwala sub-county. They were transported in a car to SEKU and acclimatized to laboratory conditions for a month. They were fed with a grower's mash (80 grams per day per chicken) until the fifth month. Layer's mash (115 grams per day per chicken) was given until the end of the experiments. Broken sorghum, green gram, and maize grains were fed in small quantities at each stage of growth and water was given adlib tum. The experimental birds were used in experiments after every two weeks rest. The cock hen ratio was maintained at 1:10. Routine management practices were performed. Replacement of the dead chicken, deworming de-toeing, debeaking, disease, and pest control was done. Only birds considered healthy using measurements of vital parameters were used for experiments. The sample size of six birds per concentration (10^{-3} - 10^{-9}g/ml) was used.

2.3 Development of ethogram

The experiments were done in an experimental chamber measuring 45cm x 45cm x 45cm. A mirror was placed behind the observation chamber to observe the eye pain behaviors from all angles. The observation chamber was opened and its relative humidity and temperature and that of the experimental room were taken using a thermo-hygrometer. A chicken was randomly selected, coded with a permanent marker, and weighed in a weighing bag using an electronic weighing balance (rating 0kg to 50kg), and the body temperature was taken electronically via the cloaca. The readings were put in a notebook and transferred into a laptop.

A wall clock was set to a zero hour in readiness for the start of the experiments. A bird from n=6 was instilled with two drops of capsaicin concentration (10^{-2}- 10^{-9}g/ml) chosen using a dropper and immediately the bird was placed into the observation chamber. There were different responses observed, counted using a counter, tallied, and noted from the chicken eye in blocks of five minutes for one hour (Kanui *et al*., 1990). The responses chosen were the most commonly seen in all the capsaicin concentrations (10^{-2}- 10^{-9}g/ml) which included eye closure, blinking, head shaking, and eye scratching. There were other responses such as neck retraction, sitting down, droppings, and vocalization which were not scored because they were not consistent and were assumed to be indirectly associated with pain due to capsaicin instillation. The procedure was followed for six birds in each concentration (10^{-2}- 10^{-9}g/ml). Controls were done using a capsaicin vehicle diluent and normal saline.

2.4 To determine the total mean time in seconds the chicken spent in painful behaviors

A chicken was randomly selected, coded with a permanent marker, and weighed in a weighing bag using an electronic weighing balance (rating 0kg to 50kg) and the body temperature was taken electronically via the cloaca. The readings were noted in a notebook.

Time taken in pain behavior was determined using a wall clock and. a stop watch. A bird from a cohort of six was instilled with two drops of capsaicin concentration (10^{-2}- 10^{-9}g/ml) with the use of a dropper. Immediately the bird was placed into the open observation chamber 45cm x 45cm x 45cm. The total time spent in pain by a chicken from the eye due to capsaicin instillation was taken using a stopwatch in blocks of five minutes for one hour. The painful responses noted were the same as seen in all the capsaicin concentrations (ethogram). The procedure was followed for six birds in a concentration range from 10^{-2} to 10^{-9}g/ml. Controls were done using a capsaicin vehicle diluent and normal saline.

2.5 Choice of the most effective capsaicin concentration

Capsaicin instillation was done as in 2.4 in a range of 10^{-2} to 10^{-9}g/ml. The time lapse between capsaicin instillation and the first response due to capsaicin instillation was taken using a stopwatch. The concentration that had the first total minimum mean responses in seconds immediately after the instillation of capsaicin was to be chosen as the most effective (Kanui *et al*., 1990).

2.6 Data analysis

The data was analyzed using Graph Pad Prism 5.0 statistical software. One-way Analysis of Variance (ANOVA) statistical function was used to check for statistical differences in the time spent on pain behaviors for different doses and control. The means were separated using Tukey's multiple comparison application.

3.0 Results

3.1The experimental birds' preparation

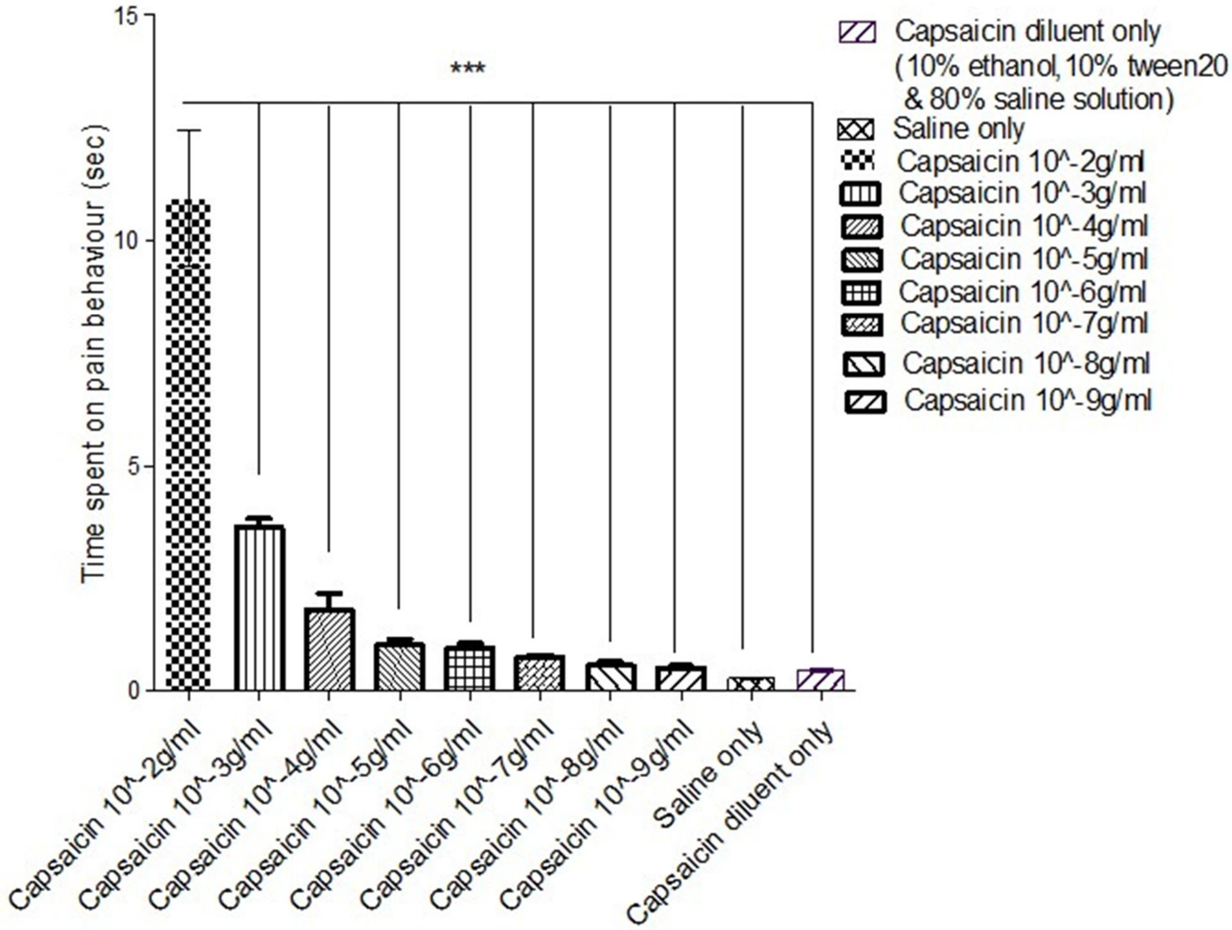
The chicken averagely weighed 0.8kg - 3.3kg with an average body (cloacal) temperature of 40.7°C - 41.6°C. The mean room temperature range was 20.7°C - 29.9°C while the mean relative humidity was 31% - 88%. At low temperatures the experimental birds had more pain relative to high temperatures. The light experimental birds experienced more pain as compared to heavy birds..

3.2 The ethogram

The painful responses demonstrated included nociception behaviors like head shaking, eye scratching, blinking, partial eye closure, and full eye closure which formed the reference behaviors used to assess pain.. There were other behavioral responses which were not scored because they were not consistent. These included neck retractions, droppings, drooping wings and vocalization.

Figure 3.1

Effects of capsaicin, capsaicin diluent, and saline on pain behavior in indigenous chicken.



Mean total responses of saline, capsaicin vehicle diluent, and capsaicin concentrations (10^{-9} – 10^{-2}g/ml).

4.0 Discussion

The induction of pain by capsaicin had been reported in humans, monkeys, cats, and rats (Kaido *et al*., 2020; Palazzo *et al*., 2010; Pelissier *et al*., 2002). Capsaicin is said to induce pain by activation of TRPV1 receptors expressed by the nociceptors (Palazzo *et al*., 2010).. The pain behaviors of the repeated capsaicin instillation had no desensitization effect. This is in agreement with findings by Kanui *et al*. (1990) which reported that capsaicin instillation in crocodiles had no desensitization effect. At the thresh hold of 10^{-9}g/ml there were pain responses a sign that the experimental birds were very sensitive to capsaicin as compared to the findings of Kanui *et al.* (1990) in capsaicin instillation onto the eye of crocodiles mentioned above. The same fact is backed by the fact that the most effective capsaicin concentration was 10^{-2}g/ml in chicken while that of crocodile was capsaicin 10^{-3}g/ml. This work was a landmark on the study of mechanisms of pain because it demonstrated that capsaicin induces pain-like behavior by activation of TRPV1 receptors expressed by nociceptors (Fattori *et al*., 2016)

Conclusion

It is evident that capsaicin potentiates responses when applied ocularly in indigenous chicken. It also emerged that in capsaicin experiments, normal saline can alternatively be used with the capsaicin diluent as control. The application of capsaicin in the eye of chicken can thus be used as a model in the assessment of pain. It is objectively confirmed in this study that the procedures used can be applied in related studies in future.

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