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Topically applied caffeine induces miosis in the ketamine/xylazine anesthetized rat

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ABSTRACT

The aim of the present study was to examine if topically applied caffeine influences pupil size in ketamine/xylazine anesthetized animals. Two experiments were carried out. In the first experiment, caffeine was topically applied to one of the eyes of 10 ketamine/xylazine anesthetized animals, while vehicle only was topically applied to the contralateral eye. In the second experiment, caffeine was topically applied to both eyes in one group of 10 ketamine/xylazine anesthetized rats, while in another group both eyes vehicle only was topically applied to both eyes. In both experiments pupil diameter was measured at 0, 10, 20, 40 and 60 min after topical application. In three of the animals, the pupil was dilated with tropicamide 5 mg/ml at 60 min after the topical application of caffeine and the pupil diameter was measured. The first experiment showed a relative miosis in caffeine treated eyes as compared to the vehicle treated eye, that changed over time. The second experiment in line with the first experiment, also showed that topically applied caffeine causes a relative miosis as compared to vehicle only that changes over time. Eyes treated with caffeine reacted with quick dilatation after tropicamide application. Topical caffeine antagonizes ketamine/xylazine anesthesia induced mydriasis in a time dependent manner.

1. Introduction

Caffeine is widely consumed in form of beverages and food, e.g. coffee, teas, many soft drinks, so-called 'energy' drinks and chocolate. Recently, it was demonstrated that topical caffeine prevents from cataract (Kronschläger et al., 2013; Varma et al., 2010). Pupil diameter may influence cataract development by regulation of the exposed area of the lens surface. There is limited information about the influence of caffeine on pupil diameter. In the literature, conflicting case reports about caffeine poisoning (Brooks, 2011; Jorens et al., 1991; Rowland and Mace, 1976) indicate both induction of miosis and mydriasis. Medscape suggests that a caffeine overdose causes mydriasis (Yew, 2013).

The pharmacokinetics of topical caffeine was recently elucidated (Kronschläger et al., 2014). Topically applied caffeine rapidly diffuses through the cornea into the anterior chamber and to the lens. The caffeine then slowly accumulates in the blood circulation to a blood concentration that is low compared to the concentration applied topically and below the blood level that would be achieved by the maximium oral intake of caffeine recommended by FDA.

The combination of ketamine and xylazine is a common injectable anesthetic in animal experiments. This combination is usually injected intraperitoneally as a single dose (Gaertner et al., 2008). Ketamine, a non-competitive NMDA receptor antagonist, provides anesthesia, analgesia and amnesia (Aroni et al., 2009). Xylazine, a α2-adrenergic agonist, supplements the effect of ketamine and induces sedation and muscle relaxation. Together they give a safe anesthesia in rodents with a wide therapeutic window without danger and without need for further monitoring than visual observation (Gaertner et al., 2008).

Besides its central effects, ketamine also impacts on the peripheral nervous system by interference with the cholinergic system. Ketamine inhibits muscarinic acetylcholine receptors causing a prominent sympathetic response including bronchodilation and mydriasis (Sinner and Graf, 2008). A mydriatic effect is also observed after pure xylazine injection. There are two mechanisms of action for mydriasis by xylazine. Xylazine centrally inhibits the parasympathetic stimulus to the iris. Further, it stimulates the α2-adrenergic receptor in the iris and CNS (Hsu, Betts and Lee, 1981a; Hsu, Lee and Betts, 1981b).

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The aim of the present study was to examine if topically applied caffeine influences pupil size in ketamine/xylazine anesthetized animals.

2. Materials and methods

The current study comprises two experiments. In the first experiment, caffeine was topically administered to one eye and placebo to the contralateral eye. Pupil size was measured. In the second experiment, caffeine or placebo was administered topically to both eyes of each animal.

2.1. Animals, drugs and measurements

Six-week-old Sprague-Dawley rats were used in the experiments. Ethical approval was obtained from the Uppsala Ethical Committee on Animal Experiments. The animals were kept and treated according to the ARVO Statement for the use of animals in Ophthalmic and Vision Research.

The caffeine eye drops were prepared by adding 0.9% of hydroxypropylmethyl cellulose (Sigma–Aldrich; H7509) to an aqueous solution of caffeine to a final concentration of caffeine of 72 mM (Kronschläger et al., 2013, 2014; Varma et al., 2010). Placebo eye drops were prepared with the same vehicle but without caffeine.

The pupil diameter was measured by comparing the pupil size with a ruler in the slit lamp microscope.

2.2. Experimental procedure

2.2.1. Experiment I

In the first experiment, the animal was anesthetized with a mixture of 94 mg/kg ketamine and 14 mg/kg xylazine, injected intraperitoneally. As soon as the anesthetized animal showed no reflexes, caffeine drops were instilled in one eye and placebo drops in the contralateral eye. The pupil diameter was measured in both darkness and in room-light luminance at regular intervals.

2.2.2. Experiment II

In the second experiment, a caffeine and a placebo treated group of animals, respectively were anesthetized with the same method as that used in the first experiment. Depending on group belonging, caffeine or placebo was topically administered to both eyes. Pupil diameter was recorded with the same procedure as in the first experiment.

2.3. Experimental design

In the first experiment, 10 animals received caffeine in one eye and placebo in the contralateral eye. Pupil diameter was measured once at regular intervals after topical administration of eye drops. The difference of pupil diameter was calculated for each animal.

In the second experiment 20 animals were randomized to either a caffeine group or a placebo group. Pupil diameter was measured once in each eye of each animal at regular intervals after eye drop application. In both experiments the measurement intervals were at 0 (immediately after drop application), 10, 20, 40 and 60 min after topical application.

In 3 of the caffeine treated animals, tropicamide was topically applied at 60 min after the initial topical caffeine application. Then, 5 min later, the pupil diameter was measured on both eyes.

2.4. Statistical parameters

The significance levels and confidence coefficients were set to 0.05 and 0.95, respectively.

3. Results

3.1. Experiment I

There was no apparent difference in pupil diameter between darkness and room light conditions in anesthetized topically treated animals. Therefore, all observations were made in room light conditions. There was a difference of pupil diameter between the placebo treated eye and the caffeine treated eye as indicated by a 95% confidence interval for the mean difference, being different from zero (CI_d (0.95 = 0.3 ± 0.2 mm; d.f. 9). The confidence interval for the mean difference was estimated using the average of all latency periods within the same eye for each of the placebo and the caffeine treated eyes, respectively.

Immediately after the topical administration of placebo drops, the pupil diameter increased slightly to a maximum around 20 min after initiation of the anesthesia and then returned to baseline (Fig. 1).

After topical administration of caffeine, the pupil diameter decreased throughout the observation period.

The difference of pupil diameter between the placebo treated eye and the caffeine treated eye within animal increased to a plateau around 40 min after the instillation of the eye drops, and then decreased (Fig. 2).

3.2. Experiment II

Macroscopically, it was observed that the pupil diameter contracts after topical application of caffeine as compared to after topical application of placebo (Fig. 3).

Statistical inference indicated no change of pupil diameter in the placebo treated animals between the latency time 0 min and the latency time 60 min ($CI_{dP:0-60}$ (0.95) = 0.3 ± 0.6 mm, d.f = 9) but a decrease in pupil diameter in the caffeine treated animals ($CI_{dC:0-60}$ (0.95) = 2.1 ± 0.5 mm, d.f. 9) and this difference in response to the treatment was different between the two groups ($CI_{dP\text{-}dC:0-60}$ (0.95) = 2.0 ± 0.8 mm, d.f. 18).

To analyze the effect of topical application of caffeine on pupil diameter over time, the average pupil diameter for both eyes within animal was averaged, for each time point, and a 95% confidence interval for the mean among animals within time was calculated for each treatment group (Fig. 4).

After initiation of the anesthesia, the pupil diameter in the placebo treated animals, tended to increase slightly and then

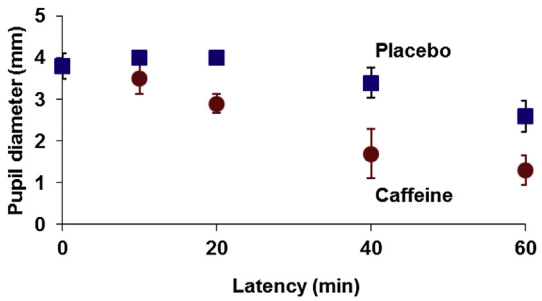

Fig. 1. Pupil diameter as a function of time after instillation of placebo in one eye (squares) and caffeine, 72 mM, in the contralateral eye, (filled circles). Bars are 95% confidence intervals for the mean (d.f. = 9).