Adenosine 5'-Monophosphate Aerosol Challenge Does Not Provoke Airflow Limitation in Healthy Cats

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Abstract

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The purpose of our study was to investigate the effects of nebulized adenosine 5'-monophosphate on airflow limitation in healthy cats determined by barometric whole body plethysmography (BWBP), in comparison to the effects of carbachol. Ten healthy 4- to 6-year-old domestic shorthair cats were included in the study. Each cat was placed in a BWBP plexiglass chamber (volume 38 l). Changes in box pressure were measured at baseline and after nebulization of vehicle and increasing concentrations of carbachol and adenosine 5'- monophosphate. Airway responsiveness was monitored as increases in enhanced pause (PENH), a unitless variable derived from dose-response curves estimating airflow limitation. The chosen endpoint was the agonist concentration which increased PENH to 300% of the value obtained after saline nebulization (PCPENH 300). Inter-day repeatability of measurements was assessed by repeated bronchoprovocations with both agonists 2-3 days apart. For carbachol, PCPENH300 was reached in all cats and correlated significantly between days (mean ± SD; 0.54 ± 0.42 mg/ml and 0.64 ± 0.45 mg/ml respectively; r = 0.58, $p < 0.05$) In contrast, we found no reaction to adenosine 5'-monophosphate even with the highest concentration nebulized during both measurements. At baseline, mean ± SD PENH was 0.47 ± 0.18 and 0.58 ± 0.24 (measurements 1 and 2), whereas PENH after 500 mg/ml adenosine 5'- monophosphate was 0.46 ± 0.20 and 0.71 ± 0.37. All bronchoprovocation tests were well tolerated by the cats. We conclude that healthy airways in cats do not demonstrate airway responsiveness to inhaled adenosine 5'- monophosphate. This is in agreement with observations in humans as well as our previous findings in dogs, where adenosine 5'- monophosphate had no effect on healthy canine airways, but caused significant airflow limitation after induction of acute bronchitis. To define the value of bronchoprovocation testing with adenosine 5'- monophosphate in the feline respiratory tract, further investigation of this agonist in cats with spontaneous lower airway disease will be required.

Aerosol challenge, adenosine 5'- monophosphate, healthy cats

Inhalational aerosol challenge with measurement of airway responsiveness is considered a valuable diagnostic tool for the detection of lower airway disease. Airway hyper-responsiveness is defined as an abnormal increase in airflow limitation following the exposure to a stimulus and is described as an important pathophysiological characteristic of lower airway diseases (Van Schoor et al. 2000). In addition to humans, this phenomenon was observed in several animal species including mice, rats, guinea pigs, cats and dogs (Chand et al. 1993; Hamelmann et al. 1997; Theodorou et al. 1997; Hofmann et al. 1999; Hannon et al. 2001).

A bronchoprovocative agonist commonly used for aerosol challenge in cats is carbachol, which causes airflow limitation by a direct action on the effector cells involved in the reduction of flow, such as airway smooth muscle cells, bronchial vascular endothelial cells and mucus producing cells. In humans, indirectly acting agonists, such as adenosine

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5'- monophosphate (AMP), are thought to be more specific, since they only cause airflow limitation in inflamed airways. Their mechanism of action is based on cells other than the effector cells; these cells subsequently interact with the aforementioned effector cells. Cells that act as an intermediary between the indirect stimuli and the effector cells are inflammatory cells (e.g. mast cells) and neuronal cells (Van Schoor et al. 2000; Currie et al. 2003). Since there is a lack of effector cells for indirect stimuli in the airways of healthy subjects, no or very weak responses are expected after their administration. In contrast, directly acting agents should cause bronchoconstriction even in subjects without evidence of respiratory inflammation (Polosa and Holgate 1997; Van Den Berge et al. 2001).

Barometric whole body plethysmography (BWBP) is an extremely non-invasive method used for monitoring airway responses to induced bronchoconstriction in rodents, cats or dogs (Chand et al. 1993; Hamelmann et al. 1997; Hofmann et al. 1999; Talavera et al 2004). Breathing pattern is assessed dynamically by analysis of box-pressure signals that increase and decrease with the respiratory cycle (Fig. 1) (Hofmann et al. 1999).
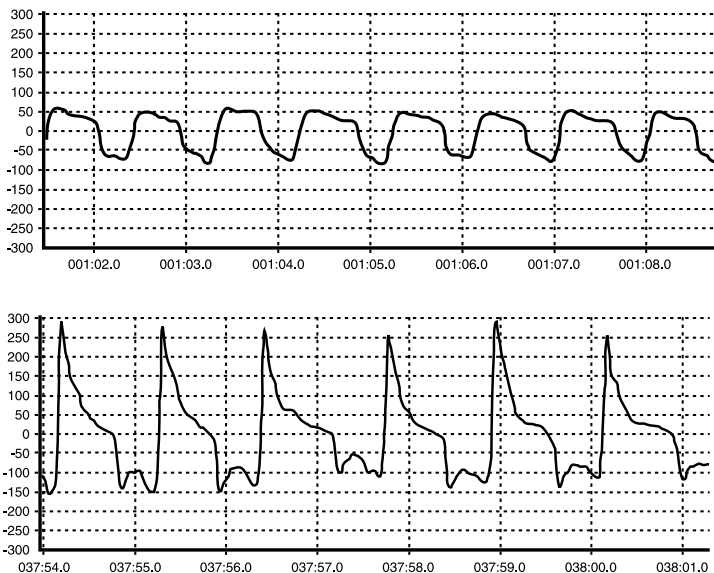

Fig. 1. An illustrative picture of box pressure signals obtained from a healthy cat by use of barometric whole body plethysmography.
1a - Signals obtained before aerosol exposure to carbachol.
1b - Signals obtained during peak bronchoconstriction after carbachol aerosol challenge.

Measurements derived by analysis of box-pressure signals change substantially with bronchoconstriction caused by different pharmacologic stimuli (e.g. histamine or metacholine challenge) (Chand et al. 1993; Hamelmann et al. 1997; Hofmann et al. 1999). Unfortunately, the pressure changes, occurring inside the closed plethysmograph chamber containing a breathing animal, are not only caused by the air flow during inspiration and expiration, but are also significantly influenced by changes in gas humidification and temperature as air moves between the box and the lungs. Since it is not possible to distinguish between these two sources of changes in box pressure, the usefulness of PENH in the assesment of lung function is accordingly to a certain degree limited (Jason et al. 2003; Adler et al 2004). Despite these limitations, BWBP is still

considered useful in clinical applications, especially because of its extreme non-invasiveness (Halley et al. 2004).

The purpose of our study was to investigate the effects of nebulized AMP on airflow limitation in healthy cats using barometric whole body plethysmography (BWBP), in comparison to the effects of carbachol. We hypothetized that carbachol, as directly acting agonist, would cause airflow limitation in healthy cats; in contrast, aerosol challenge with indirectly acting agent (AMP) should have no effects on healthy airways and cause no airflow limitation. Aerosol challenge with both bronchoprovocants might be useful in clinical practice as a non-invasive, easy to perform method for screening airway disease or monitoring the effect of drug therapy, especially in feline respiratory diseases of inflammatory origin.

Materials and Methods

Animals

Ten healthy Domestic shorthair cats ranging from 4 to 6 years (mean = 5.8) of age were included in the study. Three cats were castrated males and seven were neutered females. Animals were related and their body weight was 4 - 6 (mean = 4.86) kg. Cats in the study were kept as a colony of experimental animals at the Institute of Nutrition, Veterinary University Vienna, Austria. Their health status was regularly checked and revealed no history or clinical signs consistent with respiratory disease within 12 months of the onset of the study. All the animals were regularly vaccinated and dewormed. In all cats, a physical examination, CBC and biochemical blood analysis were performed prior to the first testing period, which revealed no abnormalities. Lateral and ventrodorsal radiographs of all the cats were examined. In nine animals, radiographs were considered as normal and in one cat a slightly increased interstitial pattern was found. All procedures were approved by the National Animal Health Care Authorities to fulfill the criteria concerning use of animals for experimental studies.

Procedure

We used a similar procedure for barometric whole body plethysmography (BWBP) to that described by Hoffman et al. (1999) in cats. Animals were placed in a BWBP chamber consisting of a plexiglass box with an inner volume of 38 l. A screen pneumotachograph was attached to the wall of the main chamber to permit dynamic assessment of pressure fluctuations in the chamber. One pole of a low-pressure differential transducer (SCXL004, Invensys Sensor Systems, Milpitas, Calif) ($\pm$ 10 cm H_2O) was open to the main chamber and the other pole was open to a reference chamber equilibrated with atmospheric pressure by way of a small channel (1.5 mm; 67% decrease in pressure during a 10 second period). Continuous bias flow (4.8 l/min) was used to maintain the oxygen concentration while simultaneously preventing CO_2 from accumulating in the chamber ($< 0.1\%$ CO_2). Transduced signals were amplified, digitalized and sampled at 100 Hz by use of commercial software (Bio System XA, version 2.7 β, BUXCO Electronics Inc, Wilmington, NC) which also provided breath-by-breath analysis of waveforms. Inclusion criteria for waveforms that were analyzed included an inspiratory volume > 15 ml, inspiratory time (Ti) > 0.15 seconds and < 10 seconds, and a ratio of inspiratory volume to expiratory volume between 0.8 and 1.2 (Hirt et al. 2003).

Calibration of the chamber pressure signal was performed dynamically by injecting 50 ml of room air via syringe into the main chamber of the BWBP and integration of the area under the resulting flow curve. Pressure signals were analyzed by computer software to obtain values for different respiratory variables, especially enhanced pause (PENH). Enhanced pause is a unitless variable derived from observations that relaxation time (time from peak expiratory pressure in chamber to a value that is 30% of that peak) decreases and peak expiratory flow/peak inspiratory flow (i.e, ratio of peak chamber pressure during expiration to peak chamber pressure during inspiration) increases during bronchoconstriction in cats (Hofmann et al. 1999), guinea pigs (Chand et al. 1993) and mice (Hamelmann et al. 1997). Values for PENH were calculated as follows: PENH = ([Te/RT] - 1) × (PEF/PIF), where Te means expiratory time, RT relaxation time, PEF peak expiratory flow and PIF peak inspiratory flow. The protocol included measurement of PENH, tidal volume (TV) and respiratory rate (RR) before and after aerosol administration of vehicle and bronchoconstrictor agonists. Each animal was allowed to acclimate to the environment of the plethysmograph box for 3-5 minutes before each measurement. Firstly, measurement without nebulization was performed to obtain baseline values; this was subsequently followed by nebulization of 0.9 % saline (post-saline challenge) and finally by nebulization of increasing concentrations of the respective bronchoprovocative agonist. Carbachol (Carbachol, Fluka Chemie Gmbh, Buchs, Switzerland) to be nebulized was prepared at concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2, 5 mg/ml (Hirt et al. 2003) and AMP (Adenosine 5'-monophosphate disodium salt, Fluka Chemie Gmbh, Buchs, Switzerland) at concentrations of 0.1, 1, 10, 100 and 500 mg/ml (Marks et al. 1996; Currie et al. 2003). Stock solutions were obtained by solving the dry powder in 0.9% saline. Dilutions were prepared with 0.9% saline and stored at a temperature of 4 °C during the whole testing period (Martínez-García et al. 2002).

PENH was used as a function of the increasing concentrations of bronchoprovocative agonists, to characterize airway responsiveness as a concentration-dependent response. Aerosol administration was performed through

a valved opening in the chamber by use of a jet nebulizer driven by a compressor (Pari Master, PARI Gmbh, Starnberg, Germany), which produced particles with a diameter of 2-3 mm on average. Aerosol administration of saline and the increasing concentrations of the different bronchoprovocative agents was performed for 1 min each, followed by a 7-min period of data acquisition, which also facilitated clearance of bronchoprovocative agent from the box. The peak value for PENH after each dose of agonist was the highest mean value for 10 consecutive breaths during the 7-min observation period. When the peak value for PENH exceeded 300% of postsaline challenge value (ie, 3 times the post-saline value) for > 10 consecutive breaths and substantial changes in respiratory pattern (e.g. increased expiratory effort) were observed clinically, additional aerosol administrations were not performed. The provocative concentration of the agonist that increased PENH to 300% of post-saline value (PCPENH300) was obtained by interpolation of the concentration-response curve between the final 2 doses of provocative agonist (Hirt et al 2003).

In order to assess the inter-day repeatability of measurements, a second bronchoprovocation with each agent was performed. Subsequent bronchoprovocation tests in individual animals were performed in 2-3 day intervals to exclude influences of prior nebulization. Cats were tested throughout the day always in the same order. Challenges with described bronchoprovocants were performed in the following order: day 0 - first carbachol challenge, day 2 - first AMP challenge, day 4 - second carbachol challenge, day 7 - second AMP challenge.

Statistical analysis

Data were statistically analyzed by ANOVA for repeated measurements using SPSS 11.5 for Windows to describe the inter-day repeatability of PCPENH300 values obtained by repeated nebulization of carbachol.

Results

For carbachol, PCPENH300 was reached in all cats and correlated significantly between days (mean ± SD; 0.54 ± 0.42 mg/ml and 0.64 ± 0.45 mg/ml respectively; $r = 0.58$, $p < 0.05$; Fig. 2). Tidal volume measured after saline and the highest concentration of carbachol being nebulized was 31.64 ± 11.11 ml and 59.71 ± 32.25 ml (mean ± SD) for the first carbachol challenge, 28.82 ± 23.63 ml and 86.00 ± 38.36 ml for the second carbachol challenge. Respiratory rate received after nebulisation of saline and the highest concentration of agonist was 49 ± 17 and 37 ± 12 breaths/minute (mean ± SD) for the first carbachol challenge.
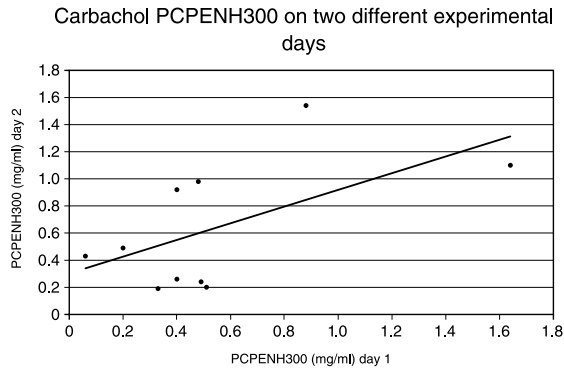

Fig. 2. The effects of nebulized carbachol on airflow limitation in the airways of 10 healthy Domestic shorthair cats by the use of barometric whole body plethysmography was investigated. Plot of carbachol PCPENH300 values (mean ± SD; 0.54 ± 0.42 mg/ml and 0.64 ± 0.45 mg/ml, respectively; $r = 0.58$, $p < 0.05$) obtained on two different experimental days. Each point represents one cat. Good agreement of measurements can be seen.

In contrast, we found no reaction to AMP even with the highest concentration nebulized during both measurements. At baseline, mean ± SD PENH was 0.47 ± 0.18 and 0.58 ± 0.24 (measurements 1 and 2), whereas PENH after 500 mg/ml AMP was 0.46 ± 0.20 and 0.71 ± 0.37. Values of TV mesured after saline and 500 mg/ml AMP nebulisation were (when expressed as mean ± SD breath/minute) 32.82 ± 24.85 and 31.90 ± 17.28 ml for the first AMP challenge, 49.44 ± 49.39 and 35.73 ± 13.24 ml for the second AMP challenge.

Respiratory rate measured after saline and 500 mg/ml AMP nebulisation were (when expressed as mean ± SD breaths/minute) 46 ± 15 and 47 ± 14 breaths/ minute for the first AMP challenge, 54 ± 14 and 49 ± 13 breaths/ minute for the second AMP challenge.

All bronchoprovocation tests were well tolerated by the cats.

Discussion

In this study, airway responsiveness to aerosol administration of carbachol and AMP in healthy cats was investigated. Using carbachol - known to be a directly acting bronchoprovocative agonist - an increase of PENH more than 300% over the baseline was obtained in all cats of this study. This observation was confirmed by repeated measurements and carbachol challenge was found to be a reliable and repeatable procedure. There was no increase over post-saline challenge PENH when AMP as an indirect agonist was nebulized. Bronchoprovocative challenge with both agents was considered safe. Despite the described limitations of BWBP (Jason et al. 2003; Adler et al. 2004), bronchoprovocation testing with carbachol can still be considered in clinical practice as an easy to perform, non-invasive method, which can be used not just for diagnostic screening, but also to monitor the effect of drug therapy in inflammatory respiratory diseases in cats. Adenosine 5'- monophosphate nebulization in cats with respiratory disease needs to be investigated to describe the clinical relevance of this bronchoprovocative agent.

Barometric whole body plethysmography (BWBP), as a non-invasive tool that allows detection of airflow limitations in cats, was introduced by Hoffman and coworkers already in 1999. Indeed it has been demonstrated in cats and other species that enhanced pause (PENH), an index of airflow limitation, can be used for the detection and quantification of obstruction of the lower airways (Hamelmann et al.1997; Hofmann et al. 1999; Hirt et al. 2003; Halley et al. 2004). Lately, studies describing uncertainity of this method have been published. In comparison with other more or less experimental methods, such as measurement of input impedance in anesthetized paralyzed and tracheostomized animals or other techniques describing changes in pulmonary resistance and dynamic compliance, BWBP is considered less reliable (Jason et al. 2003; Adler et al. 2004). The major problems are changes of pressure within the box of BWBP caused by increasing gas humidification and temperature as the animal breathes. Since it is not possible to estimate the influence of these two sources of changes in box pressure, the usefulness of PENH in the assesment of lung function is accordingly to a certain degree limited (Jason et al. 2003; Adler et al. 2004). Since the previously described more precise methods evaluating lung function require general anesthesia, they are often precluded from being applied to small animal clinical patients for repeated routine diagnostic purposes. BWBP, including measurement of airway responsiveness, is considered a promising, easy to perform method, which, despite its limitations, could add significantly to routine diagnostics in various lower airway diseases (Halley et al. 2004).

In the cats of this study, we applied a BWBP and bronchoprovocation procedure similar to that which has been described by Hoffman et al. (1999). Similarly to previous studies, an increase of PENH more than 300% over the baseline was obtained in all the cats after nebulization of increasing concentrations of carbachol (Hoffman et al. 1999; Hirt et al 2003). In our study, we did not find significant differences between PCPENH300 values for carbachol when measurements were repeatedly performed in individual animals. Carbachol has been shown to be a reliable bronchoprovocative agent for airway responsiveness testing in cats.

Tidal volume and respiratory rate, which are other important variables, were measured during bronchoprovocation testing. Comparison between post-saline TV values, and TV values in bronchoconstriction may not be valid, since TV measurements in

bronchoconstriction have been reported to be erroneously increased (Ingram and Schilder 1966). On the other hand, a drop in RR, when there is airflow limitation, is an important finding showing the increase of the expiratory effort which leads to prolongation of expiratory time and finally to a decrease in RR.

Adenosine is a purine nucleoside which may be generated in allergic inflammatory conditions upon appropriate stimulation. Once produced, adenosine is able to promote a large variety of responses in the airways, such as bronchoconstriction, plasma exsudation and increased bronchial blood flow. Furthermore, as a paracrine mediator, it contributes to various aspects of the inflammatory process (Polosa and Holgate 1997). Experimentally, adenosine was able to increase the contractile responses of canine airway smooth muscle induced by histamine (Sakai et al. 1989). Similar effects can be observed after inhalation of adenosine by subjects with lower airway disease (Rutgers et al. 1999; Rutgers et al. 2000). Cushley and co-workers (1983) first reported that adenosine provoked concentration-dependent bronchoconstriction when administered by inhalation to asthmatic subjects but not to healthy volunteers. In addition, inhalation of its related nucleotide, adenosine 5'- monophasphate, produces an almost identical effect on the airways, as it is dephosphorylated to yield adenosine (Polosa and Holgate 1997). Several other experiments demonstrated that aerosol administration of increasing concentrations of AMP caused no or a very weak bronchoconstrictor response in healthy mice, Brown Norway rats, guinea pigs or rabbits (Hannon et al. 2001; Fan and Mustafa 2002). Also in our study, as was hypothetized, we did not observe significant differences between post-saline PENH values and values after nebulization of AMP even in the highest concentration. This can be best explained by AMP's proposed mechanism of action. The occurrence of high numbers of AMP effector cells, such as mast cells, is not expected in the lower airways of healthy subjects. This fact supports the idea that indirectly acting agents (e.g. AMP) for testing airway hyperresponsiveness could be of greater value in the diagnosis of lower airway inflammatory diseases, since they might reflect the degree of inflammation more specifically than directly acting stimuli (Polosa and Holgate 1997; Van Schoor et al. 2000; Polosa et al. 2002).

In the current study we investigated differences in airway responsiveness to the two different bronchoprovocative agonists carbachol and AMP in healthy cats with the use of BWBP. Carbachol, a directly acting bronchoprovocative agonist, increased PENH to more than 300% over the baseline in all animals and was found to be reliable in causing reproducible airway responses when used as aerosol. As we hypothetized, there was no increase over baseline PENH with the indirect bronchoconstrictive agent AMP in these healthy cats. The use of AMP challenge in cats requires further research to evaluate the usefulness of this bronchoprovocative agent as a diagnostic tool in feline lower airway disease. Even if obvious limitations of BWBP have been described, still this extremely non-invasive method can be of value when used as a screening diagnostic method for certain inflammatory respiratory diseases, and for evaluation of the efficacy of drug therapy in cats.

Bronchoprovokace aerosolem adenosin 5'- monofosfátu nezpůsobuje u zdravých koček omezení průchodnosti dýchacích cest

Cílem naší studie bylo porovnat vliv adenosin 5'- monofosfátu a karbacholu na průchodnost dýchacích cest u zdravých koček při využití celotělové barometrické pletysmografie. Studie byla provedena na deseti zdravých evropských krátkosrstých kočkách ve věku 4 až 6 let. Každá kočka byla umístěna do plexisklové komory pletysmografu o objemu 38 l. Následně byly zaznamenávány změny tlaku uvnitř komory, a to nejprve bez nebulizace a poté po nebulizaci vehikula s obsahem karbacholu nebo adenosin 5'- monofosfátu ve stoupající koncentraci. Reakce dýchacích cest byla sledována jako

zvýšení tzv. prodloužené pauzy (enhanced pause, PENH), proměnné bez jednotky, kterou lze odvodit z pletysmografické křivky měnící se v závislosti na stupni omezení průchodnosti dýchacích cest. Bronchoprovokace byla ukončena, pokud určitá koncentrace agonisty (tzv. provokační koncentrace, PCPENH300) zvýšila PENH o 300 % hodnoty naměřené po nebulizaci NaCl. Za účelem potvrzení opakovatelnosti měření byly bronchoprovokační zkoušky oběma agonisty prováděny opakovaně v intervalu 2-3 dnů. Při nebulizaci karbacholu bylo u všech koček dosaženo omezení průchodnosti dýchacích cest a zjištěna signifikantní korelace hodnot PCPENH300 získaných při opakovaném měření (průměr ± směrodatná odchylka; 0.54 ± 0.42 mg/ml a 0.64 ± 0.45 mg/ml; $r = 0.58$, $p < 0.05$). Na rozdíl od karbacholu, nebulizace adenosin 5'- monofosfátu, včetně nejvyšší možné koncentrace, opakovaně nevyvolala reakci dýchacích cest. Po nebulizaci NaCl byly získány hodnoty PENH (vyjádřeno jako průměr ± směrodatná odchylka) 0.47 ± 0.18 a 0.58 ± 0.24 (měření 1 a 2), a po nebulizaci 500 mg/ml adenosin 5'- monofosfátu hodnoty 0.46 ± 0.20 a 0.71 ± 0.37. Veškeré bronchoprovokační zkoušky byly zvířaty velmi dobře tolerovány. Dýchací cesty zdravých koček nereagují na inhalaci adenosin 5'- monofosfátu. Toto zjištění je v souladu se studiemi prováděnými u lidí a také s naší studií, kdy u zdravých psů neměla inhalace adenosin 5'- monofosfátu žádný účinek, avšak u psů s vyvolanou akutní bronchitidou vedla k omezení průchodnosti dýchacích cest. Pro zhodnocení významu bronchoprovokačních testů s využitím adenosin 5'- monofosfátu u koček je nezbytný další výzkum zaměřený na účinky tohoto agonisty u koček se spontánním onemocněním dolních cest dýchacích.

Acknowledgements

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