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Tonic $GABA_A$ receptor-mediated neurotransmission in the dorsal vagal complex regulates intestinal motility in rats

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Abstract

Vagal motor outflow from the dorsal vagal complex is important in the regulation of intestinal motility. The aim of our study was to test the hypothesis that within the dorsal vagal complex, tonic $GABA_A$-receptor mediated neurotransmission modulates intestinal motility. The $GABA_A$ receptor antagonist, bicuculline (methiodide), was microinjected into the dorsal vagal complex, and the effects on small intestinal and colonic motility were investigated. Rats were anesthetized and the mean arterial pressure and heart rate were monitored. Jejunal and colonic motility were measured manometrically, and motility indices were calculated manually. Bicuculline at concentrations of 0.25 or 0.5 mM in 30 nl was microinjected bilaterally into the dorsal vagal complex through stereotaxically placed micropipettes. The injection sites were confirmed histologically using the dye Alcian Blue. Bicuculline (0.5 mM) inhibited spontaneous jejunal motility by 76.3%, colonic motility by 51.7%, mean arterial pressure by 23.3% and heart rate by 27.6%. The lower concentration of bicuculline (0.25 mM) showed no inhibitory effects on intestinal motility but decreased mean arterial blood pressure by 24.1% and heart rate by 13.6%. Bilateral cervical vagotomy attenuated the bicuculline (0.5 mM)-induced inhibition of spontaneous jejunal motility, whereas the bicuculline effect on colonic motility was unaffected. The results of this study show that $GABA_A$ receptor-mediated neurotransmission in the dorsal vagal complex is involved in autonomic integration of motility of the small intestine and colon. Furthermore, our results indicate that the dorsal vagal complex regulation of jejunal motility involves vagal outflow, whereas vagal pathways do not participate in the bicuculline-induced inhibition of colonic motility. © 1998 Elsevier Science B.V.

Keywords: Bicuculline; Vagus; Brainstem; GABA (γ-aminobutyric acid); $GABA_A$ receptor; Motility; Colon; Jejunum

1. Introduction

Anatomical and electrophysiological studies have clearly established the importance of the dorsal vagal complex of the medulla in the autonomic control of visceral function. The dorsal vagal complex includes the nucleus tractus solitarius and the dorsal motor nucleus of the vagus. Moreover, vagal reflexes are involved in the regulation of a wide variety of important physiologic functions including gastric acid secretion, pancreatic secretion and gastrointestinal motility (Harper et al., 1959; Roman and Gonella, 1987; Mayer, 1994). Activation of the dorsal vagal complex or electrical stimulation of the peripheral end of the vagus nerve to activate vagal efferent fibers, alters tonic pressure within the lower esophageal sphincter (Rossiter et al., 1991), and increases the contraction amplitude in the stomach (Pagani et al., 1987; Yoon et al., 1996), small intestine (Greenwood and Read, 1985; Roman and Gonella, 1987; Gustafsson and Delbro, 1994a,b) and colon (Roman and Gonella, 1987).

Numerous neurotransmitter substances are localized within the dorsal vagal complex and have been purported to participate in the regulation of gastrointestinal motility. Microinjection into the dorsal vagal complex of thyrotrophin-releasing hormone (Rodgers and Hermann, 1985; Hornby et al., 1989; Raybould et al., 1989; Ishikawa et al., 1988; Tache et al., 1993), oxytocin (Rodgers and Hermann, 1985; McCann and Rodgers, 1990), and pancreatic polypeptide (McTigue et al., 1993) can lead to activation of neurons in the dorsal vagal complex and elicit subsequent changes in gastric motility and gastric acid secretion.

Compared to our understanding of the actions of excitatory substances in the dorsal vagal complex on gastrointestinal function, there is limited information concerning the

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effects of neuroinhibitory substances. γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the dorsal vagal complex and may be involved in the medullary regulation of the gastrointestinal tract. Blockade of $GABA_A$ receptors in the dorsal vagal complex has been reported to alter upper gastrointestinal functions such as swallowing (Wang and Bieger, 1991), lower esophageal sphincter pressure (Wasabau et al., 1995), and motility and secretion in the stomach (Feng et al., 1990; Wasabau et al., 1995). Taken together these results imply that $GABA_A$ receptor-mediated mechanisms in the dorsal vagal complex regulate upper gastrointestinal function; however, there is no information related to the medullary GABAergic regulation of motility in either the small intestine or colon.

The hypothesis of this study was that GABAergic neurotransmission in the dorsal vagal complex might play an important role in the tonic regulation of intestinal motility. This hypothesis was tested through the measurement of changes in jejunal and colonic motility in response to blockade of $GABA_A$ receptor-mediated neurotransmission in the dorsal vagal complex.

2. Materials and methods

2.1. Animal preparation and surgical procedures

All experimental procedures were approved by the University of Oklahoma institutional animal care and use committee. Experiments were performed on male Sprague–Dawley rats (Sasco, Omaha, NE) weighing 250–350 g that were fasted overnight and allowed free access to water. The animals were anesthetized with urethane (1.25 g/kg, s.c.). The jugular vein was cannulated for intravenous (i.v.) drug administration, and a trachea cannula was implanted to maintain a patent airway. To monitor the physiological status of the animals, mean arterial pressure and heart rate were continuously monitored. Mean arterial pressure was monitored using a pressure transducer connected to a catheter inserted into the femoral artery. Heart rate was derived from the arterial pressure waveform by a cardiotachometer (Beckman Instruments 9857B, Yorbalinda, CA, USA). Signals were recorded on a Sensor Medics R612 Dynograph (Yorbalinda, CA, USA). Body temperature was maintained at 37 ± 0.5°C by a water-circulating heating pad.

In a subset of animals, preparation for vagotomy was performed by careful isolation of the cervical vagi, loose placement of 6-0 silk ligature around each vagus nerve, and ultimate section of the vagi by tightening on these ligatures.

2.2. Measurement of motility

The method for recording of intestinal motility has been previously described (Greenwood and DiMicco, 1995). In brief, following a midline laparotomy, the jejunum (~10 cm from the ligaments of Trietz) and colon (~5 cm from the cecum) were isolated. A small incision was made in both regions to remove intestinal contents and then cannulated with saline-filled polyethylene tubing (OD = 7 mm; ID = 5 mm) in an oral direction. The intestinal cannulae were secured and returned into the abdominal cavity, and the abdominal incision was sutured closed. The jejunal and colonic cannulae were connected to pressure transducers so that intrajejunal and intracolonic pressure changes representing alterations in intestinal motility could be recorded. The signals from the pressure transducers were amplified and displayed on the Sensor Medics Dynograph Recorder.

2.3. Microinjection procedure

Following completion of the surgical procedures rats were mounted in a stereotaxic frame (David Kopf Instruments, Tunjunga, CA, USA) with the nose titled down at a 45° angle. The dorsal surface of the brainstem was exposed and the tip of a multibarrel glass micropipette (A-M Systems, Everett, WA, USA) was positioned in the dorsal vagal complex at the following coordinates: 0.4 mm lateral to the midline, 0.5 mm rostral to the tip of the calamus scriptorius, and 0.6 mm ventral from the surface of the brainstem. Coordinates for placement of the micropipette was derived from the atlas of Paxinos and Watson (1986). Pipette tips were pulled and cut to an outside tip diameter between 40–60 μM. Microinjections were performed using a Picospritzer model IID (General Valve, NJ, USA). All microinjections were administered in a volume of 30 nl over 60 s. The volume administered was determined by observing the movement of the fluid meniscus in the pipette barrel using a calibrated microscope.

2.4. Experimental protocol

Following completion of the stereotaxic preparations, a 30-min interval was allowed for stabilization before the data collection. In all experiments, the stabilization period was followed by a control period when spontaneous recordings of jejunal and colonic motility, and resting arterial pressure and heart rate were collected for 10 min prior to the microinjection procedures. In the first series of experiments the responses to bilateral administration of bicuculline were investigated in animals with intact vagi. Bicuculline was microinjected into the left dorsal vagal complex and then the micropipette was rapidly repositioned to administer bicuculline into the right dorsal vagal complex. The combined microinjection procedure for bilateral administration of bicuculline was completed within 5 min. The motility and cardiovascular responses were then recorded for 10 min. In protocols where both 0.25 and 0.5 mM doses of bicuculline were administered to the same animal, the lower dose was initially administered followed by a 60-min recovery period before the higher dose of bicuculline.

To determine the role of vagal efferent pathways in mediating the bicuculline-induced alterations in jejunal and colonic motility, the effects of bilateral administration of bicuculline (0.5 mM) into the dorsal vagal complex were examined in a separate group of animals following bilateral cervical vagotomy. In this protocol bilateral vagotomy was performed after the stabilization period and 30 min before microinjection of bicuculline.

2.5. Histology

The microinjection site was marked at the end of each experiment by bilateral microinjection of 10–20 nl of 2.5% Alcian Blue. Following a 10-min interval the micropipette was removed and the animals were then perfused with saline followed by 10% buffered formalin. The brains were removed and stored in formalin. The day prior to sectioning, the brain tissue was soaked overnight in a 15% sucrose solution. The following day, brain tissue was sectioned on a freezing stage microtome in 40-μm thick sections. Brainstem sections were stained with neutral red.

2.6. Drugs

Bicuculline methiodide (bicuculline) was obtained from Research Biochemicals (Natick, MA, USA) and dissolved in artificial cerebrospinal fluid (NaCl 125 mM, KCl 3.1 mM, $NaHCO_3$ 24.5 mM, $MgCl_2$ 1 mM, $NaHPO_4$ 0.5, $CaCl_2$ 1.2, and 50 mg/l of glucose). The pH of the final solution was adjusted to 7.4.

2.7. Data analysis and statistical significance

Maximal changes in heart rate and blood pressure were measured manually from the chart recording and documented in an Excel spread sheet. Motility indices were calculated manually from the chart recording in blocks of 10 min using the following equation: Motility index = number of contractions in time period $\times$ cumulative amplitude of contractions (cmH_2O) in time period/time period (min). Motility indices were expressed as a percentage of the basal activity during the 10-min control period. All statistical analysis was performed on raw data before expression to the percentage of baseline. Statistical analysis employed multifactorial analysis for repeated measures and each variable underwent independent statistical analysis. We then performed a Tukey's protected *t*-test, a post-hoc multiple comparison test. Values are shown as mean $\pm$ S.E. The significance level was set at $P < 0.05$.

3. Results

3.1. Baseline physiological parameters

All physiological parameters were allowed to stabilize before the microinjection protocols. Under basal conditions the jejunum and colon each displayed a spontaneous pattern of motor activity. The basal jejunal motility was variable with a motility index for all experiments of 3901 $\pm$ 779 ($n = 5$). In the colon basal motility was more consistent with a mean basal motility index of 661 $\pm$ 56 ($n = 4$). Basal arterial blood pressure varied from 65 to 100 mmHg (mean baseline arterial blood pressure = 87 $\pm$ 6 mmHg). Basal heart rate varied from 330 to 388 beats/min (mean baseline heart rate = 359 $\pm$ 9 beats/min).

3.2. Effects of microinjection of bicuculline into the dorsal vagal complex

Microinjection of bicuculline (0.5 mM) into the dorsal vagal complex induced a marked and sustained inhibition of both spontaneous jejunal and colonic motility. Fig. 1 illustrates an analog recording of the inhibitory effects of microinjection of bicuculline solution into the dorsal vagal complex on jejunal and colonic motility. The onset of the inhibitory responses was observed within 30 s after completion of the bilateral microinjection of bicuculline, and the maximal inhibition occurred at approximately 3 min after completion of the microinjection procedure. As illustrated in Fig. 2 A, bicuculline (0.5 mM) induced significant inhibition of both the jejunal ($P < 0.012$; $n = 5$) and colonic ($P < 0.05$; $n = 4$) motility index. The inhibition of motility in both the jejunum and colon was characterized by decreases in both the frequency and amplitude of the contractions. A lower concentration of bicuculline (0.25 mM) had no significant effect on either jejunal or colonic motility.

Microinjection of bicuculline into the dorsal vagal complex of animals with intact vagi, decreased resting heart rate and blood pressure at both the 0.25 mM and the 0.5 mM doses. (Fig. 2B). The onset of the inhibition of the cardiovascular responses was rapid (< 5 s) after completion of the bilateral microinjection of bicuculline, and the maximal inhibition was sustained for at least 2 min.

3.3. Mechanisms of bicuculline-induced changes in intestinal and cardiovascular function

Spontaneous basal motility in the jejunum and colon was less in animals which received bilateral cervical vago-

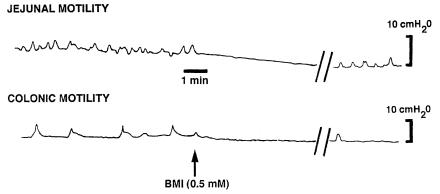

Fig. 1. Original tracing depicting the effect of microinjection of bicuculline methiodide (bicuculline: 0.5 mM) into the dorsal vagal complex on jejunal and colonic motility. The tracing following the double broken lines represent motility 25–30 min after bilateral microinjection of bicuculline into the dorsal vagal complex.

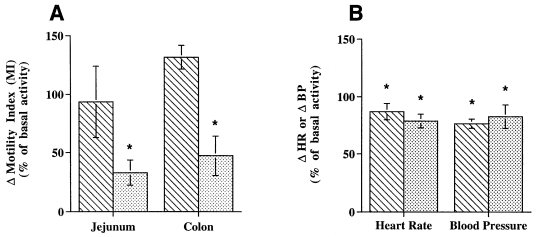

Fig. 2. Observed changes in jejunal ($n = 5$) and colonic ($n = 4$) motility index (MI) expressed as % of basal recording (A) and HR ($n = 6$) and mean BP ($n = 6$) (B) induced by bicuculline at two concentrations (0.25 mM, hatched bars; and 0.5 mM, stippled bars) microinjected into the dorsal vagal complex. In both the jejunum and colon a significant inhibition of motility was elicited by 0.5 mM bicuculline ($* P < 0.05$). At the two concentrations of bicuculline examined the bicuculline-induced bradycardia was concentration-dependent, whereas the decrease in mean arterial blood pressure showed no concentration dependent effects.

tomy compared to animals with intact vagi. In the jejunum, this 65.8% reduction in spontaneous basal motility did not reach statistical significance (vagally intact motility index $= 4107 \pm 1422$; vagotomized motility index $= 1406 \pm 563$; $P > 0.05$), whereas in the colon the 77.7% reduction in spontaneous basal motility was statistically significant (vagally intact motility index $= 692 \pm 67$; vagotomized motility index $= 154 \pm 21$; $P < 0.05$). Vagotomy elicited acute changes in arterial pressure and heart rate which occurred within 3–5 min after vagal transection but were not sustained and returned to prevagotomy levels by the end of the stabilization period.

Because bilateral microinjections of 0.25 mM bicuculline into the dorsal vagal complex were without effect on small intestinal and colonic motility, we focused our experiments on the 0.5 mM concentration of bicuculline which consistently produced a significant reduction in spontaneous baseline motility in vagally intact animals. Bilateral cervical vagotomy abolished the inhibitory effects of bicuculline (0.5 mM) on jejunal motility, however, vagotomy had no significant effect on the bicuculline-induced inhibition of colonic motility (Fig. 3). After vagotomy, bicuculline administration at 0.5 mM had no effect

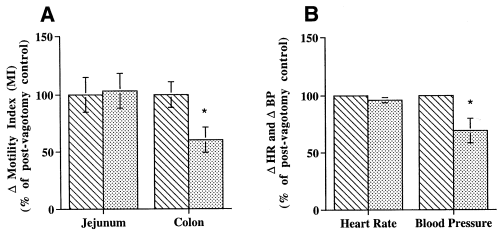

Fig. 3. Effect of bilateral cervical vagotomy on bicuculline-induced changes in jejunal ($n = 3$) and colonic motility index (MI) (A, $n = 3$) and HR and BP (B, $n = 4$). Significant differences in MI were observed in the jejunum in vagally intact (hatched bars) vs. vagotomized (stippled bars) animals ($* P < 0.05$). In the colon, vagotomy was without effect on the bicuculline-induced inhibition of motility. The bicuculline-induced bradycardia was abolished by vagotomy, whereas the bicuculline-induced decrease in BP was unaffected.

on heart rate (335 ± 27 to 322 ± 23 beats/min), whereas in animals with intact vagi, bicuculline caused a significant bradycardia (359 ± 9 beats/min to 281 ± 20 beats/min, $P < 0.05$). The depressor effect of the 0.5 mM dose of bicuculline was not inhibited by vagotomy 87 ± 6 to 69 ± 5 mmHg ($n = 5$) compared to 103 ± 10 to 68 ± 3 mmHg in vagally intact rats ($n = 4$).

4. Discussion

The results of the present investigation demonstrate that microinjection of the $GABA_A$ receptor antagonist, bicuculline, into the dorsal vagal complex, decreases spontaneous small intestinal and colonic motility. These changes in small intestinal function were accompanied by bradycardia and a decrease in mean arterial blood pressure. This finding is consistent with the hypothesis that in this region of the medulla there is tonically active GABAergic regulation of a diverse range of physiological functions including intestinal motility and cardiovascular activity.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system and GABA-containing terminals, and cell bodies have been localized within in the nucleus of the solitary tract (nucleus tractus solitarius) and the dorsal motor nucleus of the vagus (Dietrich et al., 1982; Bormann, 1988). Functionally, GABAergic mechanisms within the dorsal vagal complex are believed to modulate autonomic functions including respiration (Feldman and Smith, 1989), circulation, (Sved and Sved, 1989; Okada and Bunag, 1995) and swallowing (Wang and Bieger, 1991; Wang and Bradley, 1993). Within the dorsal vagal complex the importance of tonic GABAergic control of gastrointestinal function has not been well established, and the data that do exist are conflicting and may be due to species differences. Feng et al. (1990) observed stimulation of gastric motility and gastric acid secretion in anesthetized cats in response to microinjection of a $GABA_A$ receptor antagonist. In addition, hypothalamic $GABA_A$ receptors have been shown to influence parasympathetic outflow to the stomach (Pagani et al., 1987; Feng et al., 1990), small intestine and colon (Greenwood and DiMicco, 1995). However, there is limited information about the tonic influence of $GABA_A$ receptor activation within the dorsal vagal complex on the control of small intestinal and colonic motility.

In our experiments we determined that bicuculline, a centrally acting selective $GABA_A$ receptor antagonist, microinjected into the dorsal vagal complex, inhibits small intestinal and colonic motility. However, we were unable to define whether the effects of bicuculline occur via $GABA_A$ receptors in the nucleus tractus solitarii or dorsal motor nucleus of the vagus, due to the proximity of the sites. Moreover, although a viscerotopographic organization exists within the dorsal motor nucleus of the vagus (Berthoud et al., 1991), our microinjection procedure did

not attempt to discriminate between regions of the dorsal vagal complex. Although we have not systematically examined the effect of $GABA_A$ receptor agonists in this region on intestinal motility, we did observe that microinjection of isoguvacine, a $GABA_A$ receptor agonist, in the dorsal vagal complex produced short-lived cardiovascular responses with no changes in intestinal motility (unpublished observations). The lack of a motility effect may be due to the short duration of action of isoguvacine, however, if endogenous tonic GABAergic inhibition of the dorsal vagal complex is already maximal, addition of an exogenous $GABA_A$ receptor agonist would not be expected to have any additional effect. The concentrations of bicuculline that we selected in the current study were based on our previous studies (Barron et al., 1997). Furthermore, similar concentrations of bicuculline microinjected into the dorsomedial nucleus of the hypothalamus profoundly modulates small intestinal and colonic motility (Greenwood and DiMicco, 1995).

Careful examination of our data suggests that the effect of bicuculline on small intestinal and colonic motility follows a steep dose–response, in that 0.25 mM had no significant inhibitory effect on motility, whereas 0.5 mM caused a marked inhibition of motility. It is reasonable to speculate that if higher concentrations of bicuculline were examined they would likely abolish motility; however, the profound respiratory effects that would occur at higher concentrations of bicuculline may produce indirect effects on the gastrointestinal musculature through generalized changes in the physiology of the animal. The relatively small volumes of the injectate employed in this study and the rapid onset of the physiological changes argue for a locus of action restricted to the immediate vicinity of the injection site. In fact, moving the micropipette as little as 100 microns caused a loss of the bicuculline-induced motility and cardiovascular responses.

The hypotension and bradycardia produced by administration of bicuculline into the dorsal vagal complex are consistent with established actions of tonic activation of $GABA_A$ receptors in this region. Microinjections of bicuculline into the nucleus tractus solitarius have been shown to decrease arterial pressure and heart rate (Sved and Sved, 1989). These effects are attributable to decreased sympathetic vasoconstrictor tone and increased parasympathetic vagal efferent activity. While the cardiovascular responses due to dorsal vagal complex administration of bicuculline observed in our study are intrinsically important, the similarity of our cardiovascular findings to that of previous studies (Sved and Sved, 1989) serve to support the credibility of our novel findings related to intestinal motility.

The results of our studies aimed at elucidating the mechanisms responsible for the changes in motility clearly implicate the parasympathetic nervous system as responsible for the bicuculline-induced decrease in jejunal motility. Because bilateral cervical vagotomy abolishes the decrease in jejunal motility, we propose that the bicuculline-induced decreases in jejunal motility are mediated through vagal pathways. Although baseline colonic motility was significantly reduced by vagotomy, microinjection of bicuculline into the dorsal vagal complex produced a significant reduction in colonic motility. Therefore, the neural pathways involved in the bicuculline-induced decrease in colonic motility remain to be elucidated. The mechanism of the cardiovascular responses provides important insight into the possible mechanism(s) of the decreased motility responses induced by bicuculline. As noted in other studies (Okada and Bunag, 1995), inhibition of $GABA_A$ receptors in the nucleus tractus solitarius produces changes in arterial pressure which are, at least in part, associated with decreases in sympathetic outflow and a decreased sympathetic outflow should promote an increase in intestinal motility. It is doubtful that a decrease in sympathetic efferent activity accounts for the bicuculline-induced decrease in intestinal motility because sympathetic outflow clearly inhibits intestinal motility both in vivo (Bayliss and Starling, 1899) and in vitro; (Greenwood et al., 1987). The other component of the cardiovascular response to bicuculline administration into the dorsal vagal complex is the reduction in heart rate. This effect is attributable to an increase in vagal efferent tone since the bicuculline-induced bradycardia is virtually abolished after bilateral cervical vagotomy.

Taken together the findings of the present study suggest that GABAergic mechanisms exert a tonic inhibitory control over neurons in the dorsal vagal complex. We feel that it is reasonable to speculate that the mechanism by which a $GABA_A$ antagonist inhibits intestinal motility occurs though disinhibition of neurons in the dorsal vagal complex, thus causing an increase in the parasympathetic drive to non-adrenergic, non-cholinergic (NANC) neurons that release inhibitory neurotransmitters within the enteric plexus of the jejunum and colon. Anatomical observations in support of our findings come from work of Kirchgessner and Gershon (1989) who showed that vagal efferent axons preferentially innervate neurons in the myenteric plexus that express NANC neurotransmitters. Functionally, vagal efferent drive to the upper gastrointestinal tract can be both inhibitory and excitatory (Harper et al., 1959; Grundy et al., 1981). Since GABA acting through $GABA_A$ receptors throughout the central nervous system exerts post-synaptic inhibition (Bormann, 1988), we believe it unlikely that the decreases in jejunal and colonic motility produced by micro-injection of bicuculline into the dorsal vagal complex is caused by suppression of vagal excitatory outflow from the medulla. Rather we believe that $GABA_A$ receptor antagonism disinhibits vagal excitatory fibers that project to inhibitory neurons within the enteric nervous system to inhibit small intestinal and colonic motility.

In summary our findings demonstrate that a $GABA_A$ receptor antagonist, bicuculline, acts in the dorsal vagal complex to inhibit jejunal and colonic motility. The bicuculline-induced jejunal inhibition involves a vagal pathway

whereas other pathways are thought to mediate the inhibition of colonic motility produced by microinjection of bicuculline into the dorsal vagal complex. The present findings expand the range of physiological mechanisms influenced by activation of the dorsal vagal complex. Furthermore, our findings in the small intestine and colon complement prior findings in the esophagus and stomach, and suggest that GABAergic mechanisms in the dorsal vagal complex regulate motility throughout the entire gastrointestinal tract.

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