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RESEARCH ARTICLE

The Effects of Different Anaesthetic Agents on Electrodiagnostic Parameters in Rats

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Summary

The purpose of this study was to determine the possible effects of different anaesthetic agents on electrodiagnostic parameters in rectus abdominis, diaphragm and intercostal muscles which represent the muscles of trunk and respiratory, respectively in rat. For this purpose, ten adult, male, Sprague-Dawley rats were anaesthetized with ether, and rectus abdominis, intercostal and diaphragm muscle electromyographic (EMG) activities also with latency of n. phrenicus and n. ischiadicus were recorded in sequence by using concentric needle electrodes and surface stimulator. The same procedure was applied with xylazine/ketamine and fentanyl citrate/ketamine combinations at one week interval. Results of electrodiagnostic findings related to different anaesthetics in the same animal were compared and it was seen that parameters were different among the groups. Nerve conduction studies showed that ether and xylazine/ketamine caused significantly ($P<0.01$) prolongation of motor nerve action potential latency (MNAPL) of nervus ischiadicus. Although the prolongation of MNAPL of n. phrenicus was observed in xylazine/ketamine group, it was not significantly different between groups. There was no significant difference on the EMG activity of rectus abdominis, intercostal and diaphragm muscles following the administration of ether, xylazine/ketamine and fentanyl/ketamine. This study showed some evidence about the effects of different anaesthetics on some electrodiagnostic parameters in rats. In electrophysiological studies using anaesthetics to keep the animals immobile, it will be kept in mind that the different anaesthetics may have influence on the nerve conduction latency.

Keywords: *Rat, Elektromyography (EMG), Motor nerve action potential latency (MNAPL), Ether, Xylazine, Ketamine, Fentanyl, Anaesthesia*

Ratlarda, Farklı Anesteziklerin Elektrodiyagnostik Parametrelere Etkisi

Özet

Bu çalışmanın amacı, ratlarda göğüs ve solunum kasları olan rectus abdominis, diyafram ve interkostal kasların elektrodiyagnostik parametreleri üzerine farklı anestezik maddelerin olası etkilerini belirlemektir. Bu amaçla 10 adet yetişkin, erkek Sprague-Dawley rat, eter ile anesteziye alındı, konsantrik iğne elektrodlar ve yüzeysel uyarıcılar kullanılarak, rectus abdominis, interkostal ve diyafram kaslarının elektromyografik (EMG) aktiviteleri ile birlikte n. phrenicus ve n. ischiadicus'un latansları kaydedildi. Aynı uygulama, birer hafta arayla ksilazin/ketamin ve fentanil/ketamin kombinasyonları ile tekrarlandı. Aynı hayvanlar üzerinde farklı anesteziklerle ilgili elektrodiyagnostik bulgular karşılaştırıldı ve gruplar arasında farklılıkların olduğu görüldü. Sinir iletim çalışmaları, eter ve ksilazin/ketamin anestezilerinin n. ischiadicus'un motor sinir aksiyon potansiyeli latansında, istatistiksel olarak önemli ($P<0.01$) düzeyde uzamaya neden olduğunu gösterdi. Ayrıca ksilazin/ketamin anestezisinde n. phrenicus'un motor sinir aksiyon potansiyeli latansında da uzama gözlenmesine karşın, gruplar arasındaki fark istatistiksel olarak anlamlı bulunmadı. Eter, ksilazin/ketamin ve fentanil/ketamin anestezilerini takiben rectus abdominis, interkostal ve diaphragm kaslarının EMG aktivitelerinde istatistiksel olarak önemli bir fark belirlenmedi. Bu çalışma, ratlarda farklı anestezik maddelerin bazı elektrodiyagnostik parametrelere olan etkilerine ilgili bazı bulgular ortaya koymuştur. Hayvanları hareketsiz hale getirmek amacıyla anesteziklerin kullanıldığı elektrofizyolojik çalışmalarda, farklı anesteziklerin sinir iletim latansını etkileyebileceği akılda tutulmalıdır.

Anahtar sözcükler: *Rat, Elektromyografi (EMG), Motor sinir aksiyon potansiyeli latansı (MNAPL), Eter, Ksilazin, Ketamin, Fentanil, Anestezi*

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INTRODUCTION

Anaesthesia provides a reasonable degree of muscle relaxation so that the experimental procedure can be carried out easily and validity as well as its sufficient degree prevents the pain in animals. It is also important for animal welfare of good anaesthetic practice. On the other hand, different anaesthetic agents have different physiological effects and interfere in the results of the experiment. Therefore, suitable anaesthetic agent should be chosen according to the aim of the study and a standard anaesthesia protocol should be made in experimental studies. So that, it is of cardinal importance to know how the different anaesthetic agents effect on the physiological parameters. This knowledge is valuable both in experimental and clinical studies. In clinical practice and experimental studies, dissociative anaesthetics such as ketamine [1-3], anaesthetic combination as xylazine and ketamine [3-5], opioids such as fentanyl [3,6-8] and also inhalation agents such as ether are frequently used.

Ether has remained a popular anaesthetic in spite of safety hazard because it can be used directly in simple anaesthetic chambers [4]. High doses of fentanyl [6,8] are provided good analgesia and stable hemodynamic responses. However, the administration of fentanyl is also commonly accompanied by muscle rigidity, especially during the induction of anaesthesia [3,6,9-11] which may be associated with decrease in chest wall compliance [3,12], increase in intracranial pressure secondary to elevation in central venous pressure [13,14], and possible respiratory and acid-base disturbances [3,9,15]. Ketamine is unique in producing an unusual trance-like state known as dissociative anaesthesia [1,2] and its combination with xylazine, an alpha$_2$-adrenergic agonist with sedative and analgesic properties, results in medium planes of surgical anaesthesia [5]. Therefore, this combination is the most widely used. But, hypotensive effects of xylazine pre-dominate and this combination cause decrease in blood pressure though contrary findings [3,7].

Most of the experimental electrophysiological studies which have been carried out in anaesthetized animals show that amplitude and latency are basic data for the functional evaluation of nerve and muscle. But there are limited studies about the comparison of the possible effects of anaesthetic agents on these parameters [7,16,17]. It is pointed out that different anaesthetic agents have influence on the EMG activities depending on different muscle groups i.e. extremity or trunk muscles [7].

The purpose of this study was to determine the possible effects of different anaesthetic agents on electrodiagnostic parameters in rectus abdominis, diaphragm and intercostal muscles which represent the muscles of trunk and respiratory, respectively in rat.

MATERIAL and METHODS

In this study, 10 adult male Sprague-Dawley rats (weighing between 230-310 g) were used. Rats were housed 3 per cage and maintained on a 12:12 h light dark schedule in a temperature-controlled room (22-24°C). Rats were feed ad libitum with a standard rat diet until 24 hours before application of anaesthesia.

Three different anaesthetics; ether (within anaesthetic chamber), two combinations of ketamine (80 mg/kg/IP Alfamine®, Ege-Vet, İzmir, TÜRKİYE) with xylazine (12 mg/kg/IP; Alfazyne® Ege-Vet, İzmir, TÜRKİYE) and fentanyl citrate (15 mg/kg/IP Fentanyl citrate®, Abbott, USA) were used in the same rats. One week interval was given between anaesthetic applications due to avoid interaction within each other.

Following anaesthesia, latency of motor nerve action potential (MNAPL) studies of n. ischiadicus and n. phrenicus and electromyographic (EMG) activities of the diaphragm, rectus abdominis and intercostal muscles were performed with Nihon-Kohden Neuropack II (Tokyo, JAPAN) equipment. To study the n. ischiadicus and n. phrenicus MNAPLs, settings were designed as follows: frequency; 20 Hz to 3 KHz, gain 10 µv/div, stimulation rate: 1 Hz, duration 0.2 ms, stimulation: 5 mA. For the muscle EMG, settings of the equipment were designed as follows: frequency; 10 Hz- 5 KHz, gain 200 µv/div, analyze time: 30 msn. Concentric needle electrodes (NM-121T) and surface stimulator (ENG stimulator, SLE-5100) were utilized for record and stimulation respectively, and also band-type grounding electrode (NM-511 S) placed on the lower abdomen of the animals in all electrodiagnostic applications.

After rat was placed in right lateral recumbency, the n. ischiadicus was stimulated on caudal side of greater trochanter of femur and the response was recorded from the left gastrocnemius muscle. The n. phrenicus was stimulated on the neck and the response was recorded from the diaphragm via trans-abdominal percutaneous route. And also EMG activities of diaphragm, intercostal muscles from the 8th intercostal space and rectus abdominis were recorded.

Latency of motor nerve action potentials was defined as milliseconds (msec) and the data were analyzed statistically by using Kruskal Wallis one way analysis of variance. If there were statistically significant differences, Duncan's test was used to determine from which group the differences were originated. The mean values and standard error of means were calculated as descriptive

statistics. In electromyographic activities, the amplitudes were defined as µv and grouped into three categories. Amplitudes higher than the 60 µv labelled as “high” (H), 20-60 µv labelled as “medium” (M), and for lower than 20 µv categorized as “low” (L). Chi-square test was used for statistical analysis.

RESULTS

Percent value and statistical analysis of EMG activities in rectus abdominis, intercostal and diaphragm muscles following the ether, xylazine/ketamine and fentanyl/ketamine anaesthesia were showed in *Table 1*.

The statistical data (X±Sx) of n. ischiadicus and n. phrenicus latencies under the effects of different anaesthetics was indicated in *Table 2*.

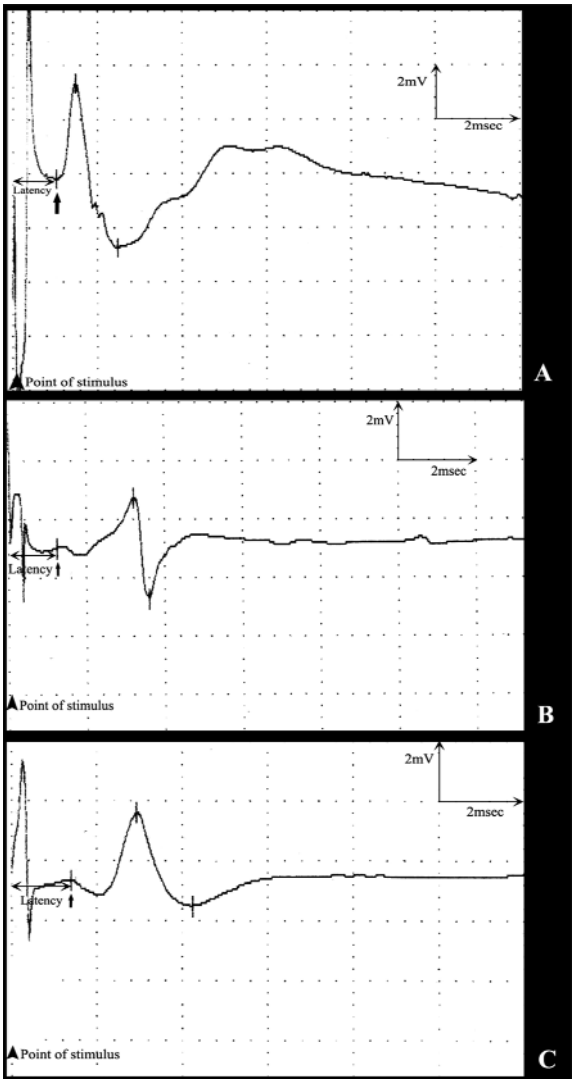

Fig 1. *Representative data from a single rat demonstrating the effects of different anaesthetics on the n. ischiadicus latency*

Şekil 1. *Farklı anesteziklerin N.ischiadicus latansına olan etkisinin temsili olarak tek bir rat üzerinde gösterilmesi*

Nerve conduction studies showed that ether and xylazine/ketamine caused significantly (P<0.01) prolongation of the MNAP latency of the n. ischiadicus *(Table 2).* Although the prolongation of MNAP latency of n. phrenicus was observed in xylazine/ketamine group, the difference between groups was not significant *(Table 2).*

Representative data from a single rat demonstrating the effects of different anaesthetics on the latency of n. ischiadicus and on the electromyographic activity of rectus abdominis muscle were given in the figures *(Fig. 1, 2).*

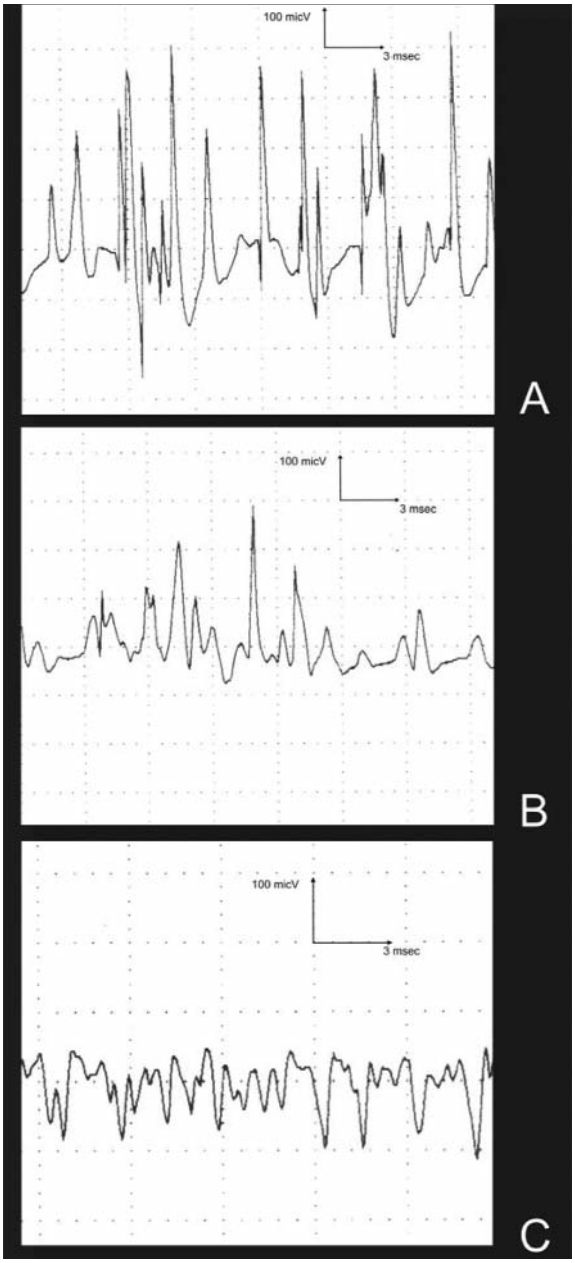

Fig 2. *Representative data from a single rat demonstrating the effects of different anaesthetics on the electromyographic activity of rectus abdominis muscle .Vertical bar; 100 µv, horizontal bar; 3 msc*

Şekil 2. *Farklı anesteziklerin M.rectus abdominis'in elektromyografik aktivitesi üzerine olan etkisinin temsili olarak tek bir rat üzerinde gösterilmesi. Dikey bar; 100 µv, yatay bar; 3 mls*

Table 1. *Percent value and statistical analysis of EMG activities (defined as low, medium and high amplitude) in rectus abdominis, intercostal and diaphragm muscles following the administration of different anaesthetics*

Tablo 1. *Farklı anestezik maddelerin uygulanmasını takiben m.rectus abdominis ile interkostal ve diyafram kaslarında saptanan EMG aktivitelerinin (düşük, orta ve yüksek amplitüd olarak tanımlanmıştır) yüzde oranları ve istatistiksel analizi*

Muscle	Ether			Xylazine/Ketamine			Fentanyl/Ketamine			X^2
	EMG Activities			EMG Activities			EMG Activities			
	Low (%)	Medium (%)	High (%)	Low (%)	Medium (%)	High (%)	Low (%)	Medium (%)	High (%)	
Rectus abdominis	20	30	50	30	50	20	20	40	40	0.725 ^{NS}
Intercostal	40	40	20	20	70	10	10	60	30	0.430 ^{NS}
Diaphragma	50	40	10	10	90	0	40	40	20	0.151 ^{NS}

N.S: *Nonsignificant* ***N.S:*** *Önemli değil*

Table 2. *The n. phrenicus and n. ischiadicus latencies following the administration of different anaesthetics*

Tablo 2. *Farklı anesteziklerin uygulanmasını takiben n. phrenicus ve n.ischiadicus latansları*

Type of Anaesthesia	Ether		Xylazine/Ketamine		Fentanyl/Ketamine		F
	n	$\bar{X}\pm S\bar{x}$	n	$\bar{X}\pm S\bar{x}$	n	$\bar{X}\pm S\bar{x}$	
Latency of n. ischiadicus	10	1.70±0.05 ^{a}	10	1.70±0.07 ^{a}	10	1.43±0.05 ^{b}	6.156 **
Latency of n. phrenicus	10	2.25±0.12	10	2.39±0.13	10	2.15±0.28	0.419 ^{NS}

*****:*** *P<0.01,* ***NS:*** *Nonsignificant, ^{a,b}: There are significant difference between groups including different letter in same column*
N.S: *Önemli değil, ^{a,b}: Aynı sütunda farklı harf içeren gruplar arasında önemli düzeyde farklılık vardır*

DISCUSSION

Surgeon expects a specimen that is motionless and has the normal vital function such as respiration during the operation of anaesthetized animal. So studies are focused on the anaesthetics agents, which provide a good muscle relaxation, though not respiration muscles. It is well known that different anaesthetics effect varying degree on EMG activities of muscles and this effect can also change depending on the muscle groups [6,7,9,10,14,16,18]. It is reported that the EMG activity increases in the gastrocnemius muscle, but no apparent alteration in the rectus abdominis muscle following the administration of fentanyl [7]. Contrary to this record, opioids are caused marked rigidity in muscles including the gastrocnemius and rectus abdominis muscles [14]. In addition to these, administration of a high dose opiate to spontaneously ventilating rats causes in an alteration of diaphragm muscle function characterized by increased expiratory EMG activity and decreased inspiratory EMG activity, which results significant reduction in tidal volume and minute ventilation [16]. The previous studies [12,16,18] reported that high-dose opiates may contribute to impaired ventilation.

In the present study, increased EMG activity of the rectus abdominis muscle in both ether and fentanyl/ketamine groups were recorded. In addition, in ether group, the EMG activity of the rectus abdominis muscle was significantly higher than xylazine/ketamine group. However there was no significant difference on the EMG activity of rectus abdominis muscle between the xylazine/ketamine and fentanyl/ketamine groups. Our results are compatible with previous reports [7,14]. In our study, EMG activity of diaphragm was recorded to be low in 4, normal in 4, and also high in 2 cases in fentanyl/ketamine group. However, the differential records of inspiratory and expiratory EMG activities of diaphragm couldn't be determined. But, in all cases generally decreases in EMG activity of diaphragm was recorded. It is proposed that the opiate-induced increases in diaphragmatic expiratory muscle activity are a consequence of an alteration in diaphragm's tension-length relationship due to abdominal muscle rigidity. The reduced diaphragm inspiratory EMG activity appears to be a consequence of profound central opiate-induced respiratory depression. And these results are correlated with the previous reports [16]. In ether group, EMG activity of diaphragm was recorded to be low in 5 cases, normal in 4, and also high in 1. Although, normal EMG activity of diaphragm was recorded in 9 cases in xylazine/ketamine group, no EMG activity was recorded to be high. Fentanyl/ketamine and ether administrations were impaired the ventilation during anaesthesia, however, xylazine/ketamine combination was not.

Campbell et al.[16] reported that the administration of a high dose opiate produced a lower increase in EMG activity in intercostal muscles than the diaphragm. In our study, the EMG activity in intercostal muscles was recorded as normal in 6 cases in fentanyl/ketamine group. These results were related with previous report [16]. Although, xylazine/ketamine combination was not influenced on the EMG activity of the intercostal muscles, more prominent differences were recorded in ether group than the other groups. These results showed that xylazine/ketamine combination did not influence the EMG activity of the diaphragm and the intercostal muscles in rats.

Latency measurement in a nerve is the objective parameters reflecting whether nerve conduction blockage exists or not. Absent or prolonged latencies show the blockage in the nerve conduction. We found prolongation of n. ischiadicus latency after both ether and xylazine/ketamine combination that is significantly ($P<0.01$) higher than the fentanyl/ketamine combination. We also clinically observed the same effect on the n. phrenicus but this was found no significant. Small sample size and relative short course of the n. phrenicus in comparison with n. ischiadicus may prevent to reach a statistically significant point.

Our results showed some evidence about the effects of different anaesthetics on some electrodiagnostic parameters in rats. There is much information about these effects and their pathophysiological mechanisms on the muscles but not on the nerves. We used a small group of animals and possibly this may have an inhibitory effect on the results, preventing to reach clear cut conclusions. But it is clearly shown that fentanyl/ketamine combination is impaired the nerve conduction parameter much more than the others, however, no side effects on the nerve conduction parameters were determined with xylazine/ketamine combination. Slower conductance in a peripheral nerve may be a sign of good anaesthesia/analgesia. But in electrophysiological studies using anaesthetics to keep the animals immobile, this prolongation of latencies is a threat to methodological correctness. This effect must be in mind and corrected after recording properly.

REFERENCES

1. White PF, Way WL, Trevor AJ: Ketamine-Its pharmacology and therapeutic uses. *Anesthesiology,* 56, 119-136, 1982.

2. Winters WD, Ferrar-Allado T, Guzman-Flores C, Alcaraz M: The cataleptic state induced by ketamine: A review of the neuropharmacology of anaesthesia. *Neuropharmacol,* 11, 303-315, 1972.

3. Flecknell P: Laboratory Animal Anaesthesia. Third edition. Academic Press San Diego, USA, 2009

4. Waynforth HB, Flecknell PA: Experimental and Surgical Technique in the Rat. Academic Press, San Diego, USA, 1994.

5. Wixson SK, White WJ, Hughes Jr HC, Marshall WK, Lang CM: The effects of pentobarbital, fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam on noxious stimulus perception in adult male rats. *Lab Anim Sci,* 37 (6): 731, 1987.

6. Bailey PL, Wilbrink J, Zwanikken P, Pace NL, Stanley TH: Anaesthetic induction with fentanyl. *Anesth Analg,* 64, 48-53, 1985.

7. Lui PW, Lee TY, Chan SHH: Fentanyl induced muscle rigidity in unanesthetized and ketamine- or thiopental anesthetized rats. *Anesthesiology,* 70, 984-990, 1989.

8. Stanley TH, Philbin DM, Coggins CH: Fentanyl-oxygen anaesthesia for coronary artery surgery: Cardiovascular and antidiuretic hormone responses. *Can Anaesth Soc J,* 26, 168-172, 1979.

9. Comstock MK, Carter JG, Moyers JR, Stevens WC: Rigidity and hypercarbia associated with high dose fentanyl induction of anesthesia. *Anesth Analg,* 60, 362, 1981.

10. Freud FG, Martin EW, Wong KC, Hornbein TF: Abdominal muscle rigidity induced by morphine and nitrous oxide. *Anesthesiology,* 38, 358-362, 1973.

11. Lai S, Lui P: Inhibition by neuropeptide Y of fentanyl-induced muscular rigidity at the locus coeruleus in rats. *Neurosci Lett,* 280 (3): 203-6, 2000

12. Scammon FL: Fentanyl-O_2 –N_2O rigidity and pulmonary compliance. *Anesth Analg,* 62, 332-334, 1983.

13. Benthuysen JL, Kien ND, Quam DD: Intracranial pressure increases during alfentanil-induced rigidity. *Anesthesiology,* 68, 438-440, 1988.

14. Benthuysen JL, Smith NT, Sanford TJ, Head N, Dec-Silver H: Physiology of alfentanil induced rigidity. *Anesthesiology,* 64, 440-446, 1986.

15. Janczewski WA, Onimaru H, Homma I, Feldman JL: Opioid-resistant respiratory pathway from the preinspiratory neurones to abdominal muscles: *In vivo* and *in vitro* study in the newborn rat. *J Physiol,* 545 (3): 1017-26, 2002.

16. Campbell C, Weinger MB, Quinn M: Alterations in diaphragm EMG activity during opiate-induced respiratory depression. *Respir Physiol,* 100, 107-117, 1995.

17. Tsou M, Lui PW, Lee TY, Pan JT, Chan S: Differential effects of prazosin and yohimbine on fentanyl-induced muscular rigidity in rats. *Neuropharmacol,* 28 (11): 1163-1168, 1989.

18. Horrow J, Abrams J, Van Ripper D, Lambson D, Storella R: Ventilatory compliance after three sufentanil-pancuranium induction sequences. *Anesthesiology,* 75, 969-974, 1991.