Effects of Propofol and Tramadol on Cholinergic Receptor-Mediated Responses of the Isolated Rat Ileum

İZOLE SIÇAN İLEUMUNUN KOLİNERJİK-RESEPTÖR-ARACILI YANITLARI ÜZERİNDE PROPOFOL VE TRAMADOLUN ETKİLERİ

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Abstract

Objective: The present study was designed to evaluate the direct effects on basal tension and its effects on the cholinergic receptor-mediated responses of propofol and tramadol in the isolated rat ileum.

Material and Methods: Full thickness segments of ileum were obtained from rats (n=8) and placed in longitudinal direction in a 10 ml organ bath. The direct effects of propofol (10^{-8}-10^{-5} M) and tramadol (10^{-6}-10^{-3} M) at basal tension and the concentration-response curves for carbachol (10^{-9}-10^{-4} M) in the presence and absence of propofol (10^{-7}-10^{-6} M) and tramadol (10^{-5}-10^{-4} M) were recorded isometrically with a Grass model 79 E polygraph.

Results: Propofol significantly reduced basal tension of ileal smooth muscle at 10^{-5} M concentration. Tramadol slightly reduced basal tension only at 10^{-3} M concentration. The contractile response of the ileum to exogenously applied carbachol was not influenced by propofol at concentration of 10^{-7} M, but was inhibited at 10^{-6} M concentration. In the presence of 10^{-6} M propofol, the concentration-response curve for carbachol was shifted to the right with significantly lower Emax and pD_2 values when compared to control values. Carbachol-induced concentration-response curve did not significantly change in the presence of 10^{-5} and 10^{-4} M tramadol.

Conclusion: Results obtained suggest that propofol reduces basal tension and cholinergic response on the rat ileum *in vitro*, but tramadol does not.

Key Words: Anesthetics, propofol, tramadol, ileum, rat

T Klin J Med Sci 2004, 24:52-56

Özet

Amaç: Bu çalışma, izole sıçan ileumunda propofol ve tramadolun bazal gerilim ve kolinerjik-reseptör-aracılı yanıtlar üzerindeki etkilerini araştırmak için planlandı.

Gereç ve Yöntemler: Wistar sıçanlardan (n=8) alınan tam tabaka ileum segmentleri 10 ml'lik organ banyolarına longitudinal şekilde yerleştirildi. Bazal gerilim üzerinde propofol (10^{-8}-10^{-5} M) ve tramadol (10^{-6}-10^{-3} M) etkilerine bakıldıktan sonra, propofol (10^{-7}-10^{-6} M) ve tramadolun (10^{-5}-10^{-4} M) varlığında ve yokluğunda karbakol'ün (10^{-9}-10^{-4} M) artan konsantrasyonlarına karşı alınan cevaplar Grass model 79 E poligraf ile izometrik olarak kaydedildi.

Bulgular: Propofol, 10^{-5} M konsantrasyonda ileal düz kasın bazal gerilimini belirgin olarak azalttı. Tramadol 10^{-3} M konsantrasyonda bazal gerilimi az miktarda azalttı. 10^{-7} M propofol varlığında karbakolün ileumdaki kasılma cevapları değişmez iken, 10^{-6} M propofolün varlığında anlamlı şekilde inhibe olmaktadır (p<0.05). 10^{-6} M propofol varlığında karbakolün konsantrasyon-cevap eğrisi, kontrol değerler ile karşılaştırıldığında belirgin olarak azalmış, Emax ve pD_2 değerleri ise sağa kaymıştır. 10^{-5} ve 10^{-4} M tramadol varlığında ise karbakolün konsantrasyon-cevap eğrisinde belirgin bir değişiklik olmamıştır.

Sonuç: Çalışmadan elde edilen bulgulara göre rat ileal düz kasında propofol bazal gerilimi ve kolinerjik cevapları azaltırken, tramadol'ün belirgin bir etkisine rastlanmamıştır.

Anahtar Kelimeler: Anestetikler, propofol, tramadol, ileum, sıçan

T Klin Tıp Bilimleri 2004, 24:52-56

Geliş Tarihi/*Received*: 15.07.2002 **Kabul Tarihi/*Accepted*:** 14.04.2003

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Gastrointestinal motility may be considerably reduced by anesthesia resulting in postoperative ileus. Propofol and tramadol are widely used as intravenous anesthetic and analgesic agents, respectively. It has been reported that they have several effects on different

smooth muscles including vascular, bronchial and gastrointestinal.[1,2] However, their effects on cholinergic function of ileal smooth muscle have not been specifically investigated. Propofol causes hypotension which is mediated by both direct vasodilation and changes in sympathetic output.[1] On the other hand, propofol was found to have no effect on bronchomotor tone and gastrointestinal motility.[2] Some experimental data obtained from pig ileum suggests that propofol produces a biphasic effect consisting of a dose-dependent contraction followed by relaxation.[3]

Tramadol, an opioid agonist and monoaminergic reuptake blocker, has been presumed to interfere less with gastrointestinal motor function compared to other opioids.[4] Reported gastrointestinal effects of tramadol include nausea, vomiting and constipation. Tramadol did not increase the baseline pressure or duration, frequency and amplitude of contraction of the bile duct sphincter in patients during endoscopic retrograde cholangiopancreatography (ERCP).[5] Tramadol was found to have a minor delaying effect on colonic transit with no effect on upper gastrointestinal transit or gut smooth muscle.[4]

Acetylcholine (Ach) is the classical excitatory neurotransmitter in the gut and it is an important regulator of gastrointestinal motility. Acetylcholine is very rapidly hydrolyzed by cholinesterase in the body. Carbachol is extremely resistant to hydrolysis by cholinesterase and has correspondingly longer durations of action.[6]

The aim of the present study was to investigate the direct effects on basal tension and effects on cholinergic receptor-mediated responses of propofol and tramadol in isolated rat ileum.

Material and Methods

Tissue Preparation

Eight male Wistar rats, weighing 250 to 300 g, were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the experiments were approved by the Cumhuriyet University-Medical Faculty, Animal Care Committee. The rats were killed by cervical dislocation. The abdomen was immediately opened and the ileum was removed and placed in previously oxygenated (95% O_2 and 5 % CO_2) Krebs' solution (composition in mM=115,48 NaCl, 4,61 KCl, 2.5 $CaCl_2$, 1.16 $MgSO_4$, 1.14 NaH_2PO_4, 21.9 $NaHCO_3$, and 10.09 glucose). Whole full thickness segments of ileum in Krebs' solution were allowed to equilibrate for 4 h at 4°C. This procedure decreases spontaneous ileal contractions and neurogenic responses, and stabilizes subsequent contractile responses to agonists. After this procedure, whole full thickness segments of ileum were placed in longitudinal direction in a 10 ml muscle bath, filled with pre-aerated Krebs solution at 37°C. The upper end of the preparation was tied to an isometric transducer (Grass FT 03, Quincy, MA,USA) and preloaded with 1-1.5 g. Tissue was allowed to equilibrate for 30 min until a stable baseline was attained.

Experimental Design

Two sets of experimental studies were performed with ileum segments obtained from eight rats. Four ileal segments were obtained from each rat. In a first series of experiments, the ileal segments were exposed to cumulative concentrations of propofol (10^{-8}-10^{-5} mol/l) or tramadol (10^{-5}-10^{-3} mol/l), respectively; a plateau response were obtained before adding each dose. The changes in basal tension were recorded on a Grass model 79 E polygraph.

In the second series of experiments, the effects of cumulatively added concentrations of alone carbachol (10^{-9}-10^{-4} mol/l) in the absence or in the presence of propofol or tramadol on ileal segments were studied. The ileum preparations were exposed to various single concentrations of propofol (from 10^{-7} to 10^{-6} mol/l) or tramadol (from 10^{-5} to 10^{-4} mol/l) 10 min prior to the addition of cumulative concentrations of carbachol (10^{-9}-10^{-4} mol/l). The number of repetition (n) stood for the number of experiments performed with tissue samples taken from different animals. All experiments were performed in a paired way.

The developed tension in response to carbachol was expressed as gram (g) and normalized for cross sectional area (CS), which was determined using the following equation.[7]

CS (mm^2)= ((tissue wet weigh (mg) / tissue lenght (mm)) x density (mg/mm^3)

A density of 1.05 was used according to Wermillon et al.[6] To evaluate the effect of carbachol, the maximum response (E max) and pD_2 values (i.e the negative logarithm of the concentration for the half-maximal response, EC_{50}) were calculated. The EC_{50} values were calculated by regression analysis of the linear portion (between 20 and 80 % of the maximum response) of the log concentration-response curves.

Drugs

The following drugs were used: carbachol (Sigma, St. Louis, MO, USA), propofol (2,6 diisopropylphenol, Aldrich Chemical Co., USA) and tramadol (trans (±) tramadol hydrochloride, ICN, Costa Mesa, CA, USA). All substances were dissolved in Krebs Ringer solution. The solutions of the drugs were freshly prepared before each experiment. The volume that added to the organ bath never exceeded 5 % of its total volume.

Statistical Analysis

Groups were compared statistically using general linear models of ANOVA followed by *Student-Newman-Keuls*-test. Differences were considered to be significant when $p<0.05$. All data are expressed as mean ± standard error of the mean (SEM).

Results

Figure 1 shows representative tracings of in vitro effect of increasing concentrations of propofol (10^{-8}-10^{-5} M) and tramadol (10^{-6}-10^{-3} M) on basal tension of ileal smooth muscle isolated from rats. The basal tension of ileum was not influenced by propofol at concentration of up to 10^{-6} M, but markedly reduced by higher concentration (10^{-5} M) ($p<0.05$). Tramadol did not alter basal tension on the ileal smooth muscle at lower concentrations (10^{-6}-10^{-4} M), but slightly reduced basal tension at 10^{-3} M concentration.

Figure 2 shows representative tracings of in vitro effect of increasing concentrations of alone carbachol(10^{-9}-10^{-4} M) and effects of increasing concentrations of carbachol in the presence of propofol (10^{-7} and 10^{-6} M) or tramadol (10^{-5} and 10^{-4} M) on ileum smooth muscle isolated from rats. Carbachol (10^{-9}-10^{-4} M) elicited concentration-dependent contraction in ileal smooth muscle (E_{max}= 4.37 ± 0.39; pD_2= 5.69 ± 0.03). Carbachol concentration-response curve was not significantly modified in the presence of 10^{-7} M propofol. However, the concentration-response curve of carbachol shifted to the right with a reduction of the maximum response (E_{max}) and pD_2 values when exposed to 10^{-6} M propofol. ($p<0.05$) (Table 1) (Fig. 3). There were no change in the corresponding pD_2 values. Carbachol-induced concentration-response curves did not significantly change in the presence of 10^{-5} and 10^{-4} M tramadol in isolated rat ileum ($p>0.05$) (Table 1) (Fig. 4). At the end of experiments, we washed out the ileal segments and the treated again with the carbachol. The concentration-response curve obtained with cumulatively increased concentrations of carbachol was not markedly different from initial curve (Fig. 1).

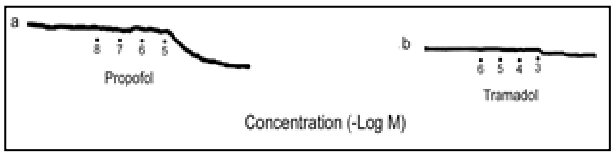

Figure 1. Original tracings of the responses elicited by different concentrations of propofol (a) and tramadol (b) on the basal tension of longitudinal ileum muscle isolated from rats.

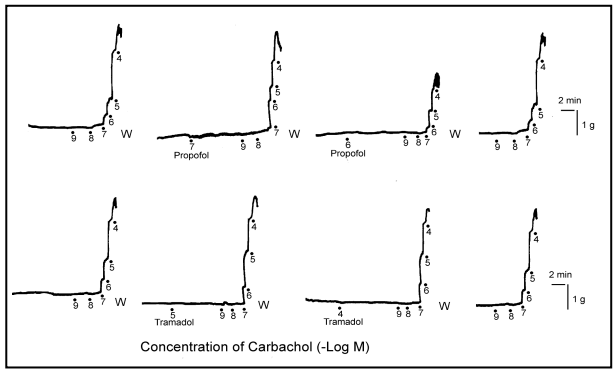

Figure 2. Original tracings of the responses elicited by different concentrations of carbachol in the presence of propofol (10^{-7} and 10^{-6} M) and tramadol (10^{-5} and 10^{-4} M) on longitudinal ileum muscle isolated from rats.

Table 1. Maksimum contraction (E_{max}) and the negative logarithm of the concentration for the half maximum response (pD_2) values of carbachol. Data are expressed as means ± SEM (n=8).

	Emax	PD2
Carbachol (control)	4.37 ± 0.39	5.95 ± 0,04
10^{-7} M propofol+carbachol	4.21 ± 0,56	5.89 ± 0,07
10^{-6} M propofol+carbachol	2.63 ± 0,25*	5.49 ± 0,03*
10^{-5} M tramadol+carbachol	4.22 ± 0,40	5.94 ± 0,05
10^{-4} M tramadol+carbachol	4.05 ± 0,35	5.91 ± 0,08

* $p< 0.05$, significantly lower compared to control group.

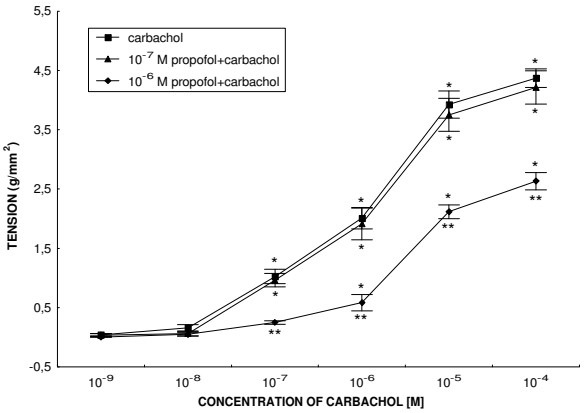

Figure 3. Concentration-response curves of carbachol in the presence of 10^{-7} and 10^{-6} M propofol on longitudinal ileum muscle isolated from rats. Data are expressed as the means ± SEM (n=8). * p<0.05 denotes significant difference within group. ** p<0.05 denotes significant difference between groups.

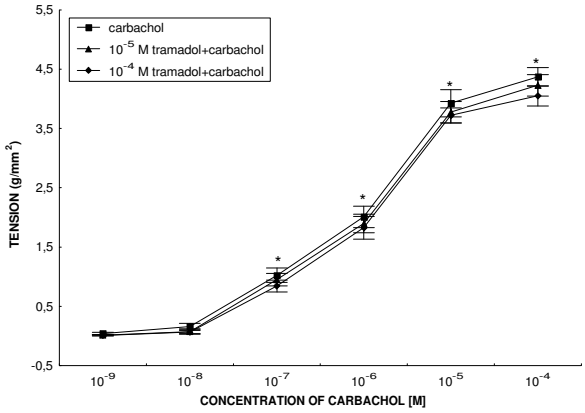

Figure 4. Concentration-response curves of carbachol in the presence of 10^{-5} and 10^{-4} M tramadol on longitudinal ileum muscle isolated from rats. Data are expressed as the means ± SEM (n=8). * p<0.05 denotes significant difference within group.

Discussion

In the present study, we showed that the basal tension of rat ileum was significantly reduced by propofol at high concentration (10^{-5} M) but not significantly affected by tramadol. We also found that propofol antagonised non-competitively the carbachol responses of the rat ileum at 10^{-6} M concentration. This effect of propofol was dose dependent, since it did not alter the carbachol-mediated contractil responses at 10^{-7} M concentration, while significantly inhibited at 10^{-6} M concentration. Tramadol did not affect carbachol-induced contractions on the rat ileum, even at high concentration (10^{-4} M). Clinically relevant concentrations of propofol and tramadol have been reported to be than $\leq 10^{-6}$ M.[5,8] The present results suggest that propofol in the clinically relevant concentrations, decrease the basal tone and cholinergic response of rat ileum, but tramadol does not.

Facilitation of the inhibitory transmission mediated by GABA seems to be related to the major mechanism of propofol anaesthesia in the central nervous system.[9] In addition to central nervous system, GABA has also been postulated as a neurotransmitter in the enteric system.[10,11] It has been shown that propofol application on the guinea-pig ileum produced a biphasic effect characterized by transient contraction and subsequent relaxation. The contractile effect was dose dependent.[3] The contraction and relaxation components of this biphasic effect are mediated by an interaction of propofol with GABA-receptors. It is already well known that GABA's contractile effect is a consequence of the activation of specific $GABA_A$-receptors located on cholinergic post-ganglionic neurons, the activation of which leads to the release of endogenous acetylcholine.[12,13] As far as the relaxing effect is concerned, this is a consequence of the activation of $GABA_B$-receptors, leading to the inhibition of the acetylcholine release.[14-16] The contractile response of the guinea-pig ileum to exogenously applied acetylcholine was not influenced by propofol at concentrations up to 7.10^{-6} M, but it was antagonised at higher concentrations of propofol.[3] In our study, propofol did not display a biphasic effect. It changed basal

tension at only high concentrations ($\geq 10^{-5}$ M) and the contractile response of the ileum to exogenously applied carbachol inhibited at only 10^{-6} M concentration of propofol. The inhibitor effect of propofol on the acetylcholine-induced contractions could be due to the activation of $GABA_B$-receptors, which leads to the inhibition of acetylcholine release. The differences between the results could be explained as different species have a different receptor density and/or different physiologic mechanisms on ileum.

Opioids, especially μ-opioid agonists, have undesirable effects on gastrointestinal system such as nausea, emesis, constipation.[17] These unwanted effects may lead to postoperative problems, in particular prolonged ileus. Effects of tramadol are distinct from those of the pure μ-opioid agonists available in clinical practice. It has a weak affinity for μ-opioid receptors.[18] Tramadol was discovered subsequently to inhibit reuptake of norepinephrine and promote release of serotonin.[19] Wilder-Smith and Bettiga showed in vivo in human that tramadol no effect upper gastrointestinal transit time.[4] Their findings seem to be consistent with our results, since tramadol did not change either basal tension or carbachol-induced contractions on the isolated rat ileum, even at supraclinical concentrations.

In conclusion, the results of present study indicate that propofol reduces the basal tension and the carbachol-induced cholinergic response of rat ileum in vitro at clinical concentrations, but tramadol has no effect at clinically relevant concentrations. Tramadol seems to have some advantages for usage postoperatively over other opioids, since it has almost no inhibitory effect on the contractility of rat ileum. Therefore, tramadol could be a useful analgesic with no interference with ileal cholinergic function.

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