Effect of Tramadol on Ovine Ureteral Smooth Muscle Contractility: an *in vitro* Experimental Study

Jamal A. Noman[1], Majid A. K. Lafi[2], Atheer K. Zgair[3]

[1]BSc, MSc, Dept. of Clinical Pharmacy, Heet General Hospital, Heet, Iraq
[2]BSc (Hons), MPhil, PhD, Dept. of Pharmacology & Toxicology, College of Pharmacy
[3]BSc, MSc, Dept. of Pharmaceutics, College of Pharmacy, Anbar University, Ramadi, Iraq

Abstract

Background: Tramadol was recently suggested to be an effective and relatively safe pharmacological treatment for pain and hyperalgesia in urinary colic from calculosis. It can apparently represent a valid therapeutic approach to this medical problem, especially in cases where conventional therapy cannot be applied. However, up to our knowledge, the *in vitro* effect of tramadol on ureteral smooth muscle contractility has not been investigated.
Objectives: The aim of this study is to investigate the effect of tramadol on the ovine spontaneous ureteral activity and attempt to determine its pharmacological basis.
Methods: *In vitro* experiments were performed on ureteral ring preparations in an organ bath. Contractions per minute (frequency) were calculated. The effect of tramadol, was obtained on its own and in the presence of naloxone, chlorpheniramine, phenoxybenzamine, atropine, or diclofenac; while, the effects of histamine, phenylephrine and acetylcholine (ACh) were recorded on their own and in the presence of their respective antagonists.
Results: Tramadol (50μM) significantly enhanced the spontaneous rhythmic motility (1.21 ± 0.25 to 3.3 ± 0.54). Further, naloxone (2μM), chlorpheniramine (1μM), atropine (1μM), or diclofenac (10μM) failed to inhibit the excitatory effect of tramadol. However, phenoxybenzamine (1μM) appreciably attenuated the excitatory effect of tramadol.
Conclusions: Tramadol produces substantial excitatory-ureteral activity by a mechanism that is still to be clarified and apparently not dependent on activation of opioid receptors, H_1-receptors, muscarinic-receptors, or prostanoid synthesis and partly dependent on aminergic mechanisms.
Key Words: Tramadol, Ureteral colic, Ovine ureteral muscle.

Corresponding author: Email:-majid_lafi@yahoo.com

Introduction

Tramadol was recently suggested to be an effective and relatively safe pharmacological treatment for pain and hyperalgesia in urinary colic from calculosis. It can apparently represent a valid therapeutic approach to this medical problem, especially in cases where conventional therapy with NSAIDs or morphine cannot be applied for problems of allergy, adverse effects or tolerance/dependence. Further, it was included as pain reliever in the guidelines of treating patients with ureteral colic [1, 2]. Furthermore, when used for treating ureteral colic, tramadol 100 mg has been shown to be as effective as pethidine 50 mg [3].

The pain of ureteral colic is thought to be related to ureteral smooth muscle spasm, oedema and inflammation at the level of the calculus, and increased peristalsis and pressure proximal to the calculus [4]. As the pain of ureteral colic is related in part to increased ureteral muscular activity, drugs able to relax the smooth muscle may be the most effective analgesics [5].

The data are conflicting for the effect of opiates on ureteral tone; results generally indicate an increase or no effect [6]. If one considers only the effects on ureteral activity, there is no basis to favour opiates in the treatment of renal colic. These agents may have ureteral spasmogenic effects that theoretically would detract from their value in the management of ureteral colic. They certainly do not have potentially valuable spasmolytic actions. Their efficacy in treating colic depends on their central nervous system actions, which decrease the perception of pain [7]. However, up to our knowledge, the *in vitro* effect of tramadol on ureteral smooth muscle contractility has not been investigated.

Materials and methods

Preparation of ureteral rings

Ovine (male, < 1 year old) kidneys with attached ureters were obtained early in the morning from the local abattoir. The specimens were transported to the laboratory within 30 minutes in chilled Krebs-Henseleit solution composed in millimolar of NaCl, 118; KCl, 4.7; $MgSO_4.7H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; $CaCl_2.2H_2O$, 2.5; glucose, 11.7. The ureters were dissected in Krebs-Henseleit solution free of connective tissue and fat. The proximal ureteral segment, 2-5 cm from the pelvis, was then isolated and 4-mm length rings were cut. From each ureter two ring-preparations were obtained and suspended vertically in 50 ml warmed and aerated (37°C; 95% O_2 and 5% CO_2) Krebs-Henseleit solution organ bath and attached to isometric force displacement transducers (Dynamometer, UFI). Tension output from the ureters was displayed on a Lectromed MX 216 two channel recorder. Initial tension of 1 g was applied to the ureters by stretch; this tension wasfound to provide optimal contractility for this preparation in previous experiments [8].

The ureter segments were then allowed to equilibrate for 30 minutes and the tension was readjusted to 1g as needed. In each experiment four ring preparations were investigated simultaneously, after a stable pattern of rhythmic contractions was established.

Experimental design

Series one experimentation

Isolated ureteral preparations were exposed to histamine (5μM), phenylephrine (10μM) or acetylcholine (ACh, 20μM); after a stable pattern of rhythmic contractions was established exposing the tissue to histamine, phenylephrine or ACh. Further**,** Interaction of histamine (5μM) with the H_1-receptor antagonist chlorpheniramine (1μM), interaction of phenylephrine (10μM) with

the α-adrenoceptor antagonist phenoxybenzamine (1μM) and interaction of ACh (20μM) with the cholinoceptor antagonist atropine (1μM); after a stable pattern of rhythmic contractions was established exposing the tissue to chlorpheniramine, phenoxybenzamine or atropine 10 minutes before recording responses to histamine, phenylephrine or ACh, respectively.

Series two experimentation

Isolated ureteral preparations were exposed to tramadol (50μM); after a stable pattern of rhythmic contractions was established exposing the tissue to tramadol. Further, Interaction of tramadol (50μM) with the opioid-receptor antagonist naloxone (2μM), the H_1-receptor antagonist chlorpheniramine (1μM), the α-adrenoceptor antagonist phenoxybenzamine (1μM) or the cholinoceptor antagonist atropine (1μM); after a stable pattern of rhythmic contractions was established exposing the tissue to naloxone, chlorpheniramine, phenoxybenzamine or atropine for 10 minutes before recording responses to tramadol.

Series threeexperimentation

In these series, tramadol (50μM) was studied in cyclooxygenase enzyme inhibitor (diclofenac) pretreated tissues; after a stable pattern of rhythmic contractions was established exposing the tissue to diclofenac (10μM) then 30 minutes later or 10 minutes **after complete** cessation of rhythmic contractions, the tissue was challenged by tramadol.

Drugs

The following drugs were used: acetylcholine chloride (Sigma), atropine sulphate monohydrate (Koch-Light Labs Ltd, UK), chlorpheniramine maleate (Chlorpheniramine Injection®,Alex Pharma), diclofenac sodium (Voltaren®; Novartis, Switzerland), histamine dihydrochloride (Sigma), naloxone hydrochloride (Naloxone®; Rotexmedica, Germany), phenoxybenzamine hydrochloride (Smith Kline & French Labs, USA), phenylephrine hydrochloride (Neo-Synephrine®; Abbott Labs, USA), tramadol hydrochloride (Trabar®; Mepha, Switzerland).

All drugs were dissolved in or diluted with distilled water before being added to the bath solution. Drugs were delivered via disposable hypodermic syringe, in volumes usually of 0.2 ml and not exceeding 0.5ml in attempt to minimize the cooling effect by the drug solution on the tissue inner bath, and to obtain the desired concentration.

Statistics

Contractions per minute (frequency) were calculated during 10 minutes period before addition of drug, and directly after the drug was added. The results were expressed as absolute values of frequency of contractions (twitch per minute). Results were expressed as means ± standard error of the mean (SEM). Statistical differences between two data sets were assessed by using the paired and unpaired Student *t*-test. When $P < 0.05$ value was considered to be significantly different.

Results

After a short latency period, isolated ureteral ring preparations obtained from the proximal ureter showed spontaneous rhythmic motility with phasic contractions and relaxation at a mean frequency of 0.98 cycles min^{-1}.

Series one experimentation

Histamine at a concentration of (5μM), phenylephrine (10μM) and ACh (20μM) all produced significant increase on the spontaneous ureteral rhythmic motility, severally. Further, after incubation of ureteral preparations with chlorpheniramine (1μM), phenoxybenzamine (1μM) or atropine (1μM) prior to challenge by histamine (5μM), phenylephrine (10μM) or ACh (20μM), respectively; in these sets, the excitatory effect of histamine, phenylephrine or ACh was abolished by

chlorpheniramine, phenoxybenzamine or atropine, respectively (Table 1).

Table 1. Effect of histamine, phenylephrine or ACh on the contractility, mean frequency per minute ± SEM, of isolated ovine ureteral preparations on their own and in the presence of their corresponding antagonists; chlorpheniramine, phenoxybenzamine or atropine, respectively, for all sets n=7.

	Effect of agonists on their own		Effect of agonists in the presence of their respective antagonists	
Drugs	**Basal activity**	**Drug effect* (%)**	**Basal activity**	**Drug effect** (%)**
Histamine (5μM)	0.94 ± 0.09	2.12 ± 0.26 (226)	0.97 ± 0.3	1 ± 0.3 (103)
Phenylephrine (10μM)	0.98 ± 0.37	1.81 ± 0.67 (185)	0.94 ± 0.03	1 ± 0.02 (106)
ACh (20μM)	0.8 ± 0.06	1.21 ± 0.1(151)	1.08 ± 0.13	1.1 ± 0.11(101)

%: percent of the respective basal activity, *P = 0.00, **P > 0.05, comparison of the respective means in these two columns

Series two experimentation

Tramadol at a concentration of (50μM) produced significant increase on the spontaneous ureteral rhythmic motility (figure 1). Further, after incubation of ureteral preparation with naloxone (2μM), chlorpheniramine (1μM), phenoxybenzamine (1μM) or atropine (1μM) prior to challenge by tramadol (50μM); in these sets, tramadol produced significant increase on the spontaneous ureteral rhythmic motility. However, phenoxybenzamine appreciably attenuated the excitatory effect induced by tramadol (Table 2).

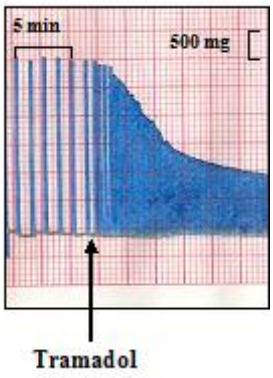

Figure 1: Traces showing the excitatory effect induced by tramadol (50μM) on the spontaneous rhythmic motility of ureteral smooth muscle.

Table 2: Effect of tramadol on the contractility, mean frequency per minute ± SEM, of isolated ovine ureteral preparations on its own (control), and in the presence of naloxone, chlorpheniramine, phenoxybenzamine or atropine, for all sets n=7.

Drugs	**Basal activity**	**Tramadol effect (%)**	***P value**
Control (without antagonist)	1.21 ± 0.25	3.3 ± 0.54** (273)	0.003
Naloxone	1.11 ± 0.24	3.2 ± 0.41 (288)	0.000
Chlorpheniramine	0.94 ± 0.13	3.82 ± 0.5 (406)	0.000
Atropine	0.78 ± 0.12	2.88 ± 0.12 (370)	0.000
Phenoxybenzamine	0.85 ± 0.03	1.16 ± 0.04** (137)	0.000

%: percent of the respective basal activity; *P value: comparison of the respective means in these two columns;**P = 0.002

Series three experimentation

After incubation of ureteral preparation with diclofenac (10μM) and cessation of spontaneous ureteral rhythmic motility prior to challenge by tramadol (50μM); in this set, tramadol significantly re-established ureteral rhythmic motility (*n* = 6).

Discussion

Ureteral smooth muscle over activity could be the main cause of ureteral colicky pain due to calculi and/or ureteral functional stasis. Therefore, relaxing ureteral smooth muscle contractility could be the target to relieve ureteral colic and/or facilitating stone propulsion[5, 9]. However, in this study tramadol (50μM) surprisingly produced a consistent increase in isolated ovine ureteral frequency. Thus, tramadol effect on the ureter *per se* could represent a controversy for its analgesic effect.

Tramadol is a synthetic codeine analogue that is a weak μ-opioid receptor agonist. Part of its analgesic effect is produced by inhibition of uptake of noradrenaline and serotonin[10].In 1993Lennon *et al* found that the excitatory effect produced by the opioid receptor agonist, morphine, on the *in vitro* canine ureter was unaffected by pre-treatment or after-treatment with the specific opiate antagonist naloxone, but was inhibited by treatment with the H_1-receptorantagonistchlorpheniramine,suggesting that the mode of action of morphine may be via histamine receptors on the ureteric smooth muscle[6]. However, in our study the excitatory effect of tramadol was not inhibited by pre-treatment with naloxone or chlorpheniramine, separately. Although, chlorpheniramine at (1μM) concentration appears to abolish effectively the excitatory effect of histamine in this study.

Further, our study has shown that phenylephrine (10μM) and ACh (20μM) also produced a consistent substantial increase in ureteral frequency that was almost completely counteracted by pretreating the tissue with the α-adrenoceptor antagonist phenoxybenzamine and the cholinoceptor antagonist atropine, respectively. It is, therefore, suggested that the excitatory effect of phenylephrine and ACh mediated by activation of α-adrenoceptors and muscarinic receptors, respectively. Similar results were reported by other investigators[11, 12]. Furthermore, pretreating the tissue with atropine failed to inhibit the excitatory effect of tramadol; thus, it is postulated that tramadol excitatory effect does not depend on muscarinic receptors activation. On the other hand, phenoxybenzamine substantially reduced tramadol induced contractility. It follows, it is reasonable to suggest that aminergic mechanisms could be involved in the modulation of this effect of tramadol. Similar lines of suggestions concerning the pharmacological actions of tramadol were made by others [10]. They suggested that tramadol inhibits neuronal reuptake of noradrenaline and serotonin.

The results of the present study demonstrate that in the isolated ovine ureter diclofenac, a non-selective COX-inhibitorwas used in a concentration of 10μM which completelyabolished spontaneous rhythmic activity of the ureteral smooth muscle.It is worth noting that the *in vitro* concentration of 10μM diclofenac mimics plasma concentration following intramuscular injection of 75 mg Voltaren®[13]. In addition, it was found that diclofenac at 10μM concentration blocked prostanoid synthesis [IC_{50} 5μM [14]], further, it is now well established that prostanoid synthesis is essential for maintaining spontaneous ureteral rhythmic motility[16,17]. It follows that after stoppage of spontaneous ureteral activity by diclofenac (10μM), herein, tramadol can produce a marked excitatory effect on

ureteral motility, which implies that prostanoids do not play an important role in this tramadol mediated effect.

In conclusion, it is suggested that this excitatory effect of tramadol mediated by a mechanism that is apparently not dependent on opioid receptors, H_1-receptors or muscarinic-receptors activation, nor prostanoid synthesis,while this action appeared to be partly mediated by aminergic mechanisms. Therefore, it is tempting to suggest that a caution should be taken when using tramadol in ureteral colicky conditions.

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