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Critical Review

Pharmacological Aspects of the Effects of Tramadol on G-Protein Coupled Receptors

Kouichiro Minami[1,2,*], Yasuhito Uezono[3], and Yoichi Ueta[4]

[1]*Department of Anesthesiology and Critical Care Medicine, Jichi Medical University, 3311-1, Yakushiji, Shimotsuke, Tochigi 329-0428, Japan*
[2]*Emergency Life-Saving Technique Academy of Tokyo, 4-5 Minamiosawa, Hachioji, Tokyo 192-0364, Japan*
[3]*Department of Pharmacology, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[4]*Department of Physiology, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishiku, Kitakyushu 807-8555, Japan*

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Abstract. Tramadol is an analgesic that is used worldwide, but its mechanisms of action have not been elucidated. It has been speculated that tramadol acts primarily through the activation of μ-opioid receptors and the inhibition of monoamine reuptake. The majority of studies to date have focused on ion channels in the central nervous system as targets of anesthetics and analgesics. During the past decade, major advances have been made in our understanding of the physiology and pharmacology of G-protein coupled receptor (GPCR) signaling. Several studies have shown that GPCRs and ion channels are targets for analgesics and anesthetics. In particular, tramadol has been shown to affect GPCRs, including muscarinic acetylcholine receptors and 5-hydroxytryptamine receptors. Here, the effects of tramadol on monoamine transporters, GPCRs, and ion channels are presented, and recent research on the pharmacology of tramadol is discussed.

Keywords: tramadol, G-protein coupled receptor, ion channel, monoamine transporter, opioid

1. Introduction

Tramadol, (1*RS*;2*RS*)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is a centrally acting analgesic and is used worldwide. It binds to μ-opioid receptors, and this is thought to be its mechanism of antinociception (1). Although it acts on μ-opioid receptors, the affinity of tramadol for the μ-opioid receptor is weak, approximately 10-fold less than that of codeine and 6,000-fold less than that of morphine. Thus, affinity alone is not sufficient to account for the analgesic action of tramadol (1, 2). The main metabolite of tramadol, *O*-desmethyl tramadol (M1), binds with about 300-fold higher affinity than the parent compound, but this is still much weaker than the affinity of morphine (1, 3). The increases in subjective and objective pain thresholds induced by tramadol contrast with those of other opioids in that they are only partially blocked by the opioid antagonist naloxone (2). Therefore, μ-opioid-receptor activation appears to be only one component of the mechanism of action of tramadol. A further mode of tramadol action has been identified as the inhibition of the reuptake of monoamines, such as norepinephrine (NE) and serotonin (5-HT), released from nerve endings. This inhibitory effect may also contribute to the analgesic effect of tramadol by inhibiting pain transmission in the central nervous system (CNS) (4, 5). Although μ-opioid receptors and monoamine transporters are thought to be the sites of tramadol activity, additional sites probably exist, based on the additional clinical and analgesic effects of tramadol.

During the past decade, major advances have been made in our understanding of the physiology and pharmacology of G-protein coupled receptor (GPCR) signaling. Studies have shown that GPCRs are targets for anesthetics and analgesics (6 – 13). However, less is

*Corresponding author.
kminami@med.uoeh-u.ac.jp, k-minami@fasd.or.jp
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known about the action of tramadol on GPCRs compared with that of NE and 5-HT transporters. Here, we review recent findings regarding the pharmacology of tramadol, highlighting GPCRs, in particular the G_q-coupled receptor and ion channel, as targets for tramadol.

2. The effects of tramadol on monoamine transporters

2.1. Inhibitory effect of tramadol on the 5-HT transporter

Tramadol has been shown to inhibit the 5-HT transporter to the same extent as the NE transporter, and several studies have suggested that the blockade of 5-HT uptake may contribute to the analgesic effect of tramadol (Table 1). Driessen and Reimann (14) reported the inhibitory effects of tramadol and M1 on 5-HT uptake in a rat brain. Tramadol inhibited the uptake of [^{3}H]-5-HT into purified rat frontal cortex synaptosomes; (dextrorotatory: +)-tramadol was about four times more potent than (levorotatory: −)-tramadol, and M1 was about ten times less potent (14). Bamigbade et al. (15) examined the actions of racemic (+/−)-tramadol, (+)-tramadol, (−)-tramadol, and M1 on 5-HT uptake in the dorsal raphe nucleus (DRN). Both (+/−)-tramadol and (+)-tramadol (each at 5 μM) significantly blocked 5-HT uptake in the DRN.

2.2. Inhibitory effect of tramadol on the NE transporter

There is evidence showing that tramadol inhibits the NE transporter as well as the 5-HT transporter (Table 1). Driessen et al. (16) reported that tramadol inhibited the uptake of [^{3}H]-NE into purified rat hypothalamic synaptosomes and showed that (−)-tramadol was about ten times more potent than (+)-tramadol. Halfpenny et al. (17) reported the effects of tramadol stereoisomers on NE uptake in the rat locus coeruleus, where only (−)-tramadol blocked the reuptake of NE and M1 was inactive. We recently reported that tramadol inhibited the desipramine-sensitive uptake of [^{3}H]-NE into bovine adrenal medullary cells (18). Although there is evidence of the inhibitory effect of tramadol on the NE transporter, the precise site of the inhibition has not been studied. Sagata et al. (18) assayed the effect of tramadol on [^{3}H]-NE uptake and [^{3}H]-desipramine binding to plasma membranes isolated from bovine adrenal medulla. Tramadol inhibited the specific binding of [^{3}H]-desipramine to plasma membranes, indicating competitive inhibition. Furthermore, atropine, hexamethonium, and naloxone, which are antagonists of muscarinic, nicotinic, and μ-opioid receptors, respectively, each caused about 15% inhibition of the specific binding of [^{14}C]-tramadol (Table 2). This suggests that tramadol may also bind to muscarinic, nicotinic, and μ-opioid receptors.

Table 1. Relative activity of monoamine transporters of (+/−)-tramadol, (+)-tramadol, (−)-tramadol, and morphine

	K_i (μM)	
	SET (μM)	NET (μM)
(+/−)-Tramadol	0.78	0.9
(+)-Tramadol	2.51	0.53
(−)-Tramadol	0.43	2.35
Morphine	No effect	No effect

Data are from refs. 14 and 15. K_i: inhibition constant, SET: 5-HT transporter, NET: norepinephrine transporter.

3. The effects of tramadol on GPCRs

As discussed above, muscarinic, nicotinic, and μ-opioid receptors are also targets of tramadol (Table 2). It would be of interest to determine whether GPCRs are the sites of the antinociception produced by tramadol. During the past decade, major advances have been made in our understanding of the physiology and pharmacology of GPCR signaling. Recent studies have shown the effects of tramadol on GPCRs; although these studies focused on G_q-coupled receptors, they also suggested that other GPCRs may also be targets for tramadol (Table 3).

Table 2. The effects of desipramine, atropine, hexamethonium, and naloxone on [^{14}C]-tramadol specific binding

	Percent of inhibition
Desipramine (10 μM)	79 ± 5
Desipramine (30 μM)	60 ± 5
Atropine (10 μM)	86 ± 7
Hexamethonium (10 μM)	84 ± 8
Naloxone (10 μM)	82 ± 10

Data are from ref. 18.

Table 3. The effects of tramadol and its metabolite M1 on G protein-coupled receptor function (IC_{50} or K_i)

	Tramadol (μM)	M1 (μM)
μ-Opioid receptor	2.1	0.0034
σ-Opioid receptor	57.6	
κ-Opioid receptor	42.7	
M_1 receptor	3.4	2
M_3 receptor	1	No effect
$5HT_{2C}$ receptor	1	
Substance P receptor	No effect	

Data are from refs. 1, 2, 3, 4, 19, and 20.

3.1. Tramadol and opioid receptors

Tramadol produced dose-related antinociception in mouse abdominal constriction, hot-plate, and tail-flick tests and in rat air-induced abdominal constriction and hot-plate tests (4). The antinociceptive activity of tramadol in the mouse tail-flick test was completely antagonized by naloxone, suggesting an opioid mechanism of action, despite the weak affinity of tramadol for the μ-opioid receptor. Tramadol bound with modest affinity to μ-opioid receptors and with weak affinity to σ and κ receptors, with K_i values of 2.1, 57.6, and 42.7 μM, respectively (4). The affinity of tramadol for μ-opioid receptors is approximately 10-fold less than that of codeine and 6000-fold less than that of morphine; therefore, affinity alone is not sufficient to account for the analgesic action of tramadol (2, 19). The metabolite M1 binds with about 300-fold higher affinity than the parent compound, but this affinity is still much lower affinity than that of morphine. In in vitro receptor binding and synaptosomal uptake experiments, (+)-tramadol was shown to be specific for the μ-opioid receptor site. The antinociceptive activity of the metabolite M1 showed a pronounced μ-selectivity (1, 3). The increases in the subjective and objective pain thresholds induced by tramadol differ from those of other opioids in that they are only partially blocked by naloxone (20). Therefore, the activation of μopioid receptors appears to be only one component of the action mechanism of tramadol.

3.2. Tramadol and muscarinic receptors

Muscarinic receptors are involved in various neuronal functions in the CNS and autonomic nervous system (21). Recent molecular cloning studies have revealed the existence of five subtypes of muscarinic receptors, M_1 – M_5 (22). Many of the muscarinic responses in peripheral tissues have been thoroughly studied using pharmacological techniques. However, relatively little is known about the functional roles of individual muscarinic receptor subtypes in the CNS. Muscarinic receptors are thought to be one of the sites of anesthetic action (6). Several recent studies have revealed that M_1 receptors may be the site of action of general anesthetics and analgesics and play an important role in their actions in the CNS (11). However, the mechanism by which tramadol inhibits musacrinic receptors has not been clarified.

Information on the effects of tramadol on muscarinic receptors is scarce. In a rat brain binding experiment, Frink et al. (3) showed that tramadol and its metabolite M1 have no affinity for M_1 receptors. We investigated the effects of tramadol on M_1 receptors in two different systems, a *Xenopus laevis* oocyte expression system and cultured bovine adrenal medullary cells. Tramadol competitively inhibited acetylcholine (ACh)-induced currents in oocytes expressing the M_1 receptor (23). In cultured bovine adrenal medullary cells, tramadol suppressed muscarine-induced cyclic GMP accumulation and inhibited the specific binding of [^{3}H]-quinuclidinyl benzilate (QNB) (23). These findings suggest that tramadol inhibits muscarinic receptor function via QNB-binding sites.

We also investigated the effects of tramadol on M_3 receptors using the *Xenopus* oocyte expression system (24). Tramadol inhibited ACh-induced currents in oocytes expressing the M_3 receptor and the specific binding of [^{3}H]-QNB, suggesting that tramadol inhibits M_3 receptor function via QNB-binding sites. This may explain the modulation of neuronal function and the anticholinergic effects of tramadol in clinical situations. To confirm the anticholinergic action of tramadol, we investigated the effects of tramadol on the pH of gastric juices during anesthesia in order to determine whether tramadol inhibits the secretion of gastric juices from gastric glands (9). After anesthesia was induced, the gastric pH was measured using pH test paper; then tramadol (100 mg), famotidine (20 mg), or saline was injected into the deltoid muscle. The gastric pH was increased by the same amount in both the tramadol and famotidine groups at 3 h after drug administration, suggesting that tramadol inhibits the secretion of gastric acid. The effects of the metabolite M1 on M_1- and M_3-receptor functions in the *Xenopus* oocyte expression system have been reported (25); the inhibitory effects of M1 on muscarinic receptors are different from those of tramadol. M1 inhibits M_1 receptor function but has little effect on M_3 receptor function (25).

3.4. Tramadol and 5-HT receptors

The neurotransmitter 5-HT is essential for many physiological processes, including the regulation of vascular and non-vascular smooth muscle contractions; modulation of platelet aggregation; and regulation of appetite, mood, anxiety, wakefulness, and perception (26, 27). To mediate this astonishing array of functions, no fewer than 15 separate receptors have evolved, of which all but two (5-HT_{3A} and 5-HT_{3B}) are GPCRs (26, 27).

Seven different families of 5-HT receptors (5-HTRs) have been identified. Several investigators recently studied the effects of tramadol on two types of metabotropic 5-HTRs. In the rat brain, tramadol and M1 have no affinity for 5-HT_{1A}, 5-HT_2, or 5-HT_3 (3). In contrast, Oliva et al. reported that the antinociceptive effect of tramadol in the mouse formalin test was mediated by the serotonergic component and that this effect was

mediated by 5-HT_2R (28). We used a whole-cell voltage clamp to test the direct effect of tramadol and M1 on 5-HT-induced Ca^{2+}-activated Cl^- currents mediated by 5-HT_{2C}R expressed in *Xenopus* oocytes and found that tramadol inhibited the 5-HT-induced Cl^- currents at pharmacologically relevant concentrations (29, 30). We also showed that tramadol inhibited the specific binding of [^{3}H]-5-HT to 5-HT_{2C}R expressed in *Xenopus* oocytes (29, 30). These results suggest that tramadol and M1 inhibits 5-HT_{2C}R function and that the mechanism of this inhibitory effect may involve competitive displacement of 5-HT binding to the 5-HT_{2C}R. Little information exists on the effects of tramadol and M1 on the other subtypes of 5-HTRs, and thus such studies are needed.

3.5. Tramadol and substance P receptors

Neuropeptide substance P receptors (SPRs) in the GPCR family are widely distributed in the CNS and peripheral nervous system (PNS). Substance P, a neurotransmitter, is released from C-fibers within nociceptive primary afferent neurons to the spinal cord and mediates part of the excitatory synaptic input to nociceptive neurons at this level (31 – 33). A recent study in mice lacking the SPR gene showed that the mice had altered pain sensitivity; nociceptive responses to certain somatic and visceral noxious stimuli were reduced in SPR knockout mice (34 – 36). Accordingly, much attention has been paid to the role of SPR in anesthetic mechanisms. Recently, we reported the inhibitory effects of the volatile anesthetics halothane, isoflurane, enflurane, and diethyl ether, as well as ethanol, on SPR (10). Moreover, the intravenous anesthetics ketamine and pentobarbital inhibit SPR-induced currents at pharmacologically relevant concentrations, whereas propofol and tramadol have little effect on the currents (37). Therefore, the antinociceptive property of tramadol would not be mediated via SPR function.

4. The effects of tramadol on receptor ion channels

The ion channels in the CNS have received a great deal of attention as targets of anesthetics and analgesics.

Table 4. The effects of tramadol and its metabolite M1 on ion channel (IC_{50} or K_i)

	Tramadol (μM)	M1 (μM)
Nicotine acetylcholine receptor	7.4	
$GABA_A$ receptor	No effect	
Glycine receptor	No effect	No effect
NMDA receptor	16.4	16.5

Data are from refs. 48 and 51.

Although the effects of tramadol on the ion channels have not been well studied, its effects on nicotinic ACh receptors, *N*-methyl-D-aspartate (NMDA) receptors, and $GABA_A$ receptors have been reported (Table 4).

4.1. Effect of tramadol on nicotinic ACh receptor ion channels

Several investigators have found that the peripheral administration of ACh, which is the classical neurotransmitter for activating nicotinic as well as muscarinic receptors, activates nociceptors (38 – 40). As mentioned above, tramadol inhibits muscarinic M_1- and M_3-receptor functions (23, 41), suggesting that cholinergic receptors could be a site of tramadol action. In capsaicin-sensitive neurons, including primary afferents and dorsal root ganglia, nicotinic as well as muscarinic ACh receptors (AChRs) are present (42), and these nociceptive pathways express some subtypes of the nicotinic AChR (43). On the other hand, nicotinic AChR-targeted compounds, such as ABT-594, have recently been shown to be potential analgesic agents (44). Little information is presently available regarding the effect of tramadol on nicotinic AChRs, but recent studies have investigated the effects of tramadol on AChRs using cultured bovine adrenal chromaffin cells (45). Shiraishi et al. used the two-electrode voltage clamp technique to investigate the effects of tramadol on nicotinic AChRs (α7 receptors) expressed in *Xenopus* oocytes (45). Tramadol suppressed carbachol-induced catecholamine secretion in a concentration-dependent manner, but had little effect on veratridine- or high K^+-induced catecholamine secretion. Tramadol also suppressed increases in the nicotine-induced $[Ca^{2+}]_i$ in a concentration-dependent manner and inhibited nicotinic currents carried by α7 receptors expressed in *Xenopus* oocytes. However, the results do not exclude the involvement of other subclasses of nicotinic AChRs in the tramadol-induced inhibition of nicotinic currents and catecholamine secretion. The inhibitory effects of tramadol on nicotinic AChR functions may be one of the antinociceptive mechanisms of tramadol.

4.2. Effect of tramadol on NMDA receptors

In addition to its essential metabolic role, glutamate is a major mediator of excitatory signals in the CNS and is involved in many physiological and pathological processes such as excitatory synaptic transmission, synaptic plasticity, cell death, stroke, and chronic pain (46, 47). Glutamate exerts its signaling role by acting on glutamate receptors, including NMDA, AMPA/kainate, and metabotropic glutamate receptors, located on pre- and post-synaptic membranes and at extra-synaptic sites. Tramadol was shown to have a little inhibitory effect on

NMDA receptors (48). In a binding experiment using the rat brain, tramadol and M1 were found to have no affinity for NMDA receptors (3).

4.3. Effect of tramadol on GABA receptors and glycine receptors

Tramadol was found to have no effect on GABA-induced currents in *Xenopus* oocytes expressing $GABA_A$ receptors (48). Tramadol and M1 metabolite did not have any effects on glycine receptors (48). Moreover, in adrenal chromaffin cells, tramadol had little effect on the currents carried by the other ligand-gated ion-channels or $GABA_A$ receptors. These results are consistent with the finding that tramadol has little hypnotic effect on patients in a clinical situation (49), unlike propofol, benzodiazepines, and barbiturates.

5. Effect of tramadol on voltage-dependent ion channels

Limited information exists on the effects of tramadol on voltage-dependent ion channels. Tramadol concentrations of 10 and 100 μM suppressed carbachol-induced catecholamine secretion in a concentration-dependent manner, but tramadol had little effect on veratridine- or high K^+-induced catecholamine secretion (23). These results indicate that tramadol reduces catecholamine secretion by inhibiting nicotinic AChRs without influencing voltage-dependent Ca^{2+} channels or voltage-dependent Na^+ channels.

6. The future prospects of research on mechanisms of actions

6.1. The relationship between tramadol and antinociception

The role of GPCRs in antinociception and analgesic actions has been investigated with a variety of approaches to date. It is reported that 5-HT can activate nociceptors (50, 51). In the contrast, several investigators have demonstrated that intrathecal serotonin induced antinociception in a variety of different animal species (52, 53). Moreover, peripheral administration of nicotine is known to activate nociceptors (54, 55) and several lines of evidence have shown that muscarinic agonists enhance antinociceptive effects, which are blocked by pretreatment with M_1, M_2, or M_3 muscarinic receptor-antagonists (56). In contrast, inhibition of the muscarinic signaling pathway induced by reducing ACh levels, inhibiting ACh release, or administering scopolamine in rat brains decreases the minimum alveolar anesthetic concentration of inhaled anesthetics (57). Several anesthetics, such as isoflurane, depress M_1- and M_3-receptor function (13, 58). As shown above, the role of the GPCRs, such as serotonin and muscarine, signaling in pain sensation is still controversial and further studies will be required to define the relationship between antinociception and GPCRs receptor functions. It would be necessary to carefully study the effects of tramadol on G-protein coupled receptors as analgesics.

6.2. The effects of tramadol on GPCRs except G_q-coupled receptors

Previous studies on the effects of anesthetics on tramadol activity examined G_q-coupled receptors such as M_1 receptor. There is little information on G_s- and G_i-coupled receptors. Recently, it is reported that halothane suppresses muscarinic receptor-mediated GTPase activity in rat atrial membranes, with an EC_{50} of 0.3 mM (59). This suggests that M_2 receptor is affected by anesthetics; this effect could be investigated using a recombinant system. Although G_q-coupled receptor signaling has been useful for studying the effects of tramadol in the *Xenopus* oocyte expression system (7, 8, 10, 23, 24, 29, 30), an analysis of G_i-coupled receptors is difficult because of the lack of an appropriate analytical method. Conklin's group recently reported an assay system for measuring the increase in $[Ca^{2+}]_i$ induced by G_i-coupled receptors using chimeric $G_{i/q}$ proteins that enable G_i protein-coupled receptors to couple to the phospholipase C pathway and increase $[Ca^{2+}]_i$ in cultured mammalian cell lines, including Chinese hamster ovary cells (60). Based on those results, we are now studying whether G_i-coupled receptors, including M_2 receptor, can elicit Ca^{2+}-activated Cl^- currents via the activation of a chimeric $G_{i/q}$ protein in *Xenopus* oocytes co-expressing G_i-coupled receptors. This system using chimeric G proteins should be useful for studying the effects of tramadol on G_s- and G_i-coupled receptors.

Although GPCRs are the most numerous therapeutic targets known, the ligands for approximately two-thirds of these receptors remain unknown. The challenge in the post-genomic era is to evaluate the roles of these orphan GPCRs (oGPCRs) in normal and diseased states and to develop new therapies based on this information. Many oGPCRs are expressed in the brain, suggesting the existence of unidentified neurotransmitters. Previous reports have shown that tramadol affects the function of GPCRs, indicating that some oGPCRs are targets of tramadol. Several oGPCRs involved in pain and nociception have been reported (61). Majane and Yang (61) reported that neuropeptide FF (NPFF) modulated pain sensation and morphine analgesia under normal and pathological conditions via both spinal and brain mechanisms (62). Studying the effects of tramadol on oGPCRs involved in pain modulation would be interesting.

6.3. The effects of (−)-tramadol and (+)-tramadol on GPCRs

The main activity of (−)-tramadol is the inhibition of NE reuptake, whereas (+)-tramadol interacts with μ-opioid receptors and increases the 5-HT concentration at the synapse via a mechanism similar to that of norepinephrine (19). The overall activity of tramadol is the sum of the specific actions of its enantiomers and its metabolite M1. The latter is characterized by its greater affinity for the μ receptor; thus, it is primarily responsible for the opiate activity (Table 1). These findings suggest that tramadol is a racemic mixture of two enantiomers that exert different pharmacological actions. Few studies have compared the effects of (−)-tramadol and (+)-tramadol on GPCRs, and this warrants further investigation.

7. Conclusion

Although much attention has been paid to the μ-opioid receptor and monoamine uptake in the CNS as targets for tramadol, several studies have shown that some GPCRs and ligand-gated ion channels are also targets for tramadol (Fig. 1). The G_s- and G_i-coupled receptors might also be targets for tramadol. More information about oGPCRs may help to elucidate the role of GPCRs in the mechanisms of tramadol activity.

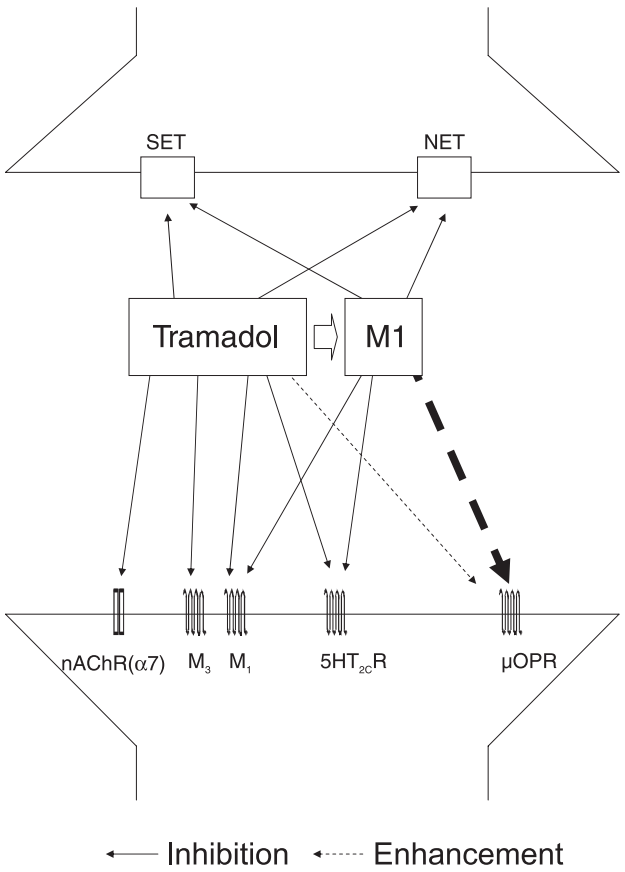

Fig. 1. The summary of the effects of tramadol on GPCR, amine transporter, and ion channels. SET: 5-HT transporter, NET: norepinephrine transporter, nAChR(α7): nicotinic acetylcholine receptor ion channnels (α7), M_1: muscarinic receptor type 1, M_3: muscarinic receptor type 3, $5HT_{2C}R$: 5-HT 2C receptor, μOPR: μ-opioid receptor.

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