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TRAMADOL SAFETY – CHOLINERGIC SYSTEM OF RAT BRAIN
WITHOUT NOCICEPTION

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Abstract: In the present investigation, changes in the levels of acetylcholinesterase (AChE) activity, acetylcholine (ACh) content, and the activity levels of plasma (PChE) and erythrocyte (EChE) cholinesterases as representatives of pseudocholinesterases were examined in different areas of the rat brain during the administration of the synthetic opioid analgesic drug tramadol (Ultram) without induction of pain. Male adult Wistar rats weighing 150 ± 20 g were used. Tramadol was injected subcutaneously (*s.c.*) into the rats at 0, 24 and 48 h, and the changes in the above cholinergic parameters were recorded after the completion of 3, 6, 12, 24, 48 and 72 h. Following administration of single dose (for rats sacrificed at 24 h) and multiple doses (for rats sacrificed at 48 and 72 h) of tramadol, the ACh content showed an increase in all brain areas. Concurrently, the AChE activity was found to decrease in all the areas. PChE and EChE showed higher activity levels, with EChE showing a higher level of activity than PChE. The levels of all the parameters examined returned towards the control levels by about 24 h after the administration of single dose of tramadol. However, the ACh levels showed an elevation at 48 and 72 h (following double and triple doses, respectively). The AChE activity levels also showed a simultaneous increase at 48 and 72 h, presumably to balance the increase in ACh levels on longer treatment with tramadol. The observed changes in the cholinergic segment presumably do not cause any physiological lesion since they reverted to control levels after the time limit of change under tramadol influence. This observation indicates that tramadol can be administered safely both under nociceptive and non-nociceptive conditions.

Keywords: ACh, AChE, PChE, EChE, rat brain areas, no-pain, tramadol

Opioids are well established in the treatment of chronic pain and are increasingly used in non-malignant pain conditions. They are broad-spectrum analgesics with potent pain-relieving qualities but also with potential adverse effects related to both short-term and long-term therapy. Opioid effects are mediated by central and peripheral opioid receptors (1). They are well tolerated in selected patients with chronic and malignant pain (2). Opioids constitute a very effective class of drugs employed for the treatment of moderate to severe pain (3, 4). Numerous studies have demonstrated that many experimental pain models are sensitive analgesic assays (5).

Tramadol hydrochloride (1RS, 2RS)-2-[[dimethylamino)methyl]-1-(3-methoxyphenyl)-cyclohexanol HCl) is a centrally acting analgesic with documented antinociceptive effects in animal models (6) and in man (7). The effects of tramadol, which has a combined opioid and monoaminergic mechanism of action, and morphine, which is a prototypical opioid, were tested in rat models of neuropathic and nociceptive pain (8). Tramadol is endowed with opioid, noradrenergic and serotonergic properties. However, various data suggest that, in addition to its analgesic effect, tramadol may have antidepressant and anxiolytic-like effects (9).

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It was reported that tramadol produces dose-related antinociception in mouse abdominal constriction, hot-plate, and tail-flick tests and in rat air-induced abdominal constriction and hot-plate tests (6). 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 (6). 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 (3, 10–12). 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 (13). Therefore, the activation of μ -opioid receptors appears to be only one component of the action mechanism of tramadol.

The effects of tramadol were studied on cholinergic muscarinic receptors. Muscarinic receptors are involved in various neuronal functions in the CNS and autonomic nervous system (14). Molecular cloning studies have revealed the existence of five subtypes of muscarinic receptors, M1–M5 (15). Studies have revealed that M1 receptors may be the site of action of general anesthetics and analgesics and may play an important role in their actions in the CNS (16). However, the mechanism by which tramadol inhibits muscarinic receptors has not been clarified. In a rat brain binding experiment, Frink et al. (17) showed that tramadol and its metabolite M1 have no affinity for M1 receptors. The effects of tramadol on M1 receptors in two different systems, a *Xenopus laevis* oocyte expression system and cultured bovine adrenal medullary cells were investigated. Tramadol competitively inhibited acetylcholine (ACh)-induced currents in oocytes expressing the M1 receptor (18). In cultured bovine adrenal medullary cells, tramadol suppressed muscarine-induced cyclic GMP accumulation and inhibited the specific binding of [3 H]-quinuclidinyl benzilate (QNB) (18). These findings suggest that tramadol inhibits muscarinic receptor function *via* QNB-binding sites. The effects of tramadol on M3 receptors using the *Xenopus* oocyte expression system (19) were also reported. Tramadol inhibited ACh-induced currents in oocytes expressing the M3 receptor and the specific binding of [3 H]-QNB, suggesting that tramadol inhibits M3 receptor function *via* QNB-binding sites. This may explain the modulation of neuronal function and the anti-cholinergic

effects of tramadol in clinical situations. The effects of the metabolite M1 on M1- and M3-receptor functions in the *Xenopus* oocyte expression system have been reported (20). The inhibitory effects of the metabolite M1 on muscarinic receptors are different from those of tramadol. M1 inhibits M1-receptor function but has little effect on M3-receptor function (20).

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 (21). To mediate this astonishing array of functions, no fewer than 15 separate receptors have evolved, of which all but two (5-HT3A and 5-HT3B) are GPCRs (22). Investigators have examined the effects of tramadol on two types of metabotropic 5-HTRs. In the rat brain, tramadol and M1 have no affinity for 5-HT1A, 5-HT2, or 5-HT3 (17). In contrast, Oliva et al. (23) 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-HT2R (23).

There is also evidence showing that tramadol inhibits the norepinephrine (NE) transporter as well as the serotonin (5-HT) transporter. Driessen et al. (24) 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. (25) 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. It was recently reported that tramadol inhibited the desipramine-sensitive uptake of [3 H]-NE into bovine adrenal medullary cells (26). Although there is evidence for the inhibitory effect of tramadol on the NE transporter, the precise site of inhibition has not been identified. Sagata et al. (26) 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. This suggests that tramadol may also bind to muscarinic, nicotinic, and μ -opioid receptors.

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 (12). 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. These findings suggest that tramadol is a racemic mixture of two enantiomers that exert different pharmacological actions. It binds to μ -opioid receptors, and this is thought to be its mechanism of antinociception (11). Thus, 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 NE and 5-HT, released from nerve endings (27). This inhibitory effect may also contribute to the analgesic effect of tramadol by inhibiting pain transmission in the CNS (6, 28). 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.

With regard to the cholinergic segment, earlier findings indicated that high concentrations of acetylcholine (ACh), choline acetyltransferase (CAT) and acetylcholinesterase (AChE) in the thalamus of the brain are suggestive of some important cholinergic pathways. In general, there is a good correlation between ACh content, CAT and cholinesterase (especially AChE) activities, but it is found to be poor in the dorsal root fibers of cerebellum (29). In several areas, the AChE and CAT activities are very well correlated with exceptionally high level in striatum, interpeduncular nucleus, and hippocampus (30–32). Studies have suggested the role of cholinergic mechanisms in the modulation of pain (33–36).

In view of the role of cholinergic system as a segment of the multiple transmitter systems of the brain, the present study was undertaken to examine the changes in this segment in different areas of the rat brain following the administration of the opioid analgesic tramadol. While changes would be logically expected during pain situations, the interest of the present study is to examine the possible changes during non-induction of pain under administration of single and multiple subcutaneous doses of tramadol with reference to different timeframes. Thus, this investigation was carried out under the premise that the actions of analgesic drugs would be the same in the presence or absence of pain, and to explore the accompanying biochemical changes in different areas of the brain.

MATERIALS AND METHODS

Procurement and maintenance of experimental animals

Male adult Wistar rats weighing 150 ± 20 grams were used as the experimental animals. The rats were purchased from the Indian Institute of Science (I.I.Sc.), Bangalore, India, and maintained in polypropylene cages under laboratory conditions of $28 \pm 2^\circ\text{C}$, LD 12 : 12 photoperiod and 75% relative humidity. The rats were given standard pellet diet and water *ad libitum*. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA (Regd. No. 438/01/a/CPCSEA/dt.17.07.2001) in its resolution number 15 /IAEC/SVU/2001/dt. 4.03.2008).

Selection of the drug

Tramadol, the drug selected for the present investigation, was chosen with reference to the earlier work (6) by Raffa RB. Although there are extensive preclinical, clinical, post-marketing and epidemiological data indicating relatively low – but not zero – abuse/dependence, questions continue to arise about its abuse potential and appropriate regulatory classification. There is nothing unique about tramadol in this regard, but its multimodal mechanism of action, pharmacologically active enantiomers, and active metabolite make it a particularly instructive and relevant example for our current work.

Tramadol (Ultram) was obtained as a commercial grade chemical from Apollo Pharmacy, Hyderabad, India. It is marketed as a racemic mixture of the (1R, 2R)- and (1S,2S)- enantiomers with a weak affinity for the μ -opioid receptor (approximately 1/6000th that of morphine) (37). The (1R, 2R)-(+)-enantiomer is approximately four times more potent than the (1S, 2S)-(-)-enantiomer in terms of μ -opioid receptor affinity and 5-HT reuptake, whereas the (1S, 2S)-(-)-enantiomer is responsible for noradrenaline reuptake effects (38). These actions appear to produce a synergistic analgesic effect, with (1R,2R)-(+)-tramadol exhibiting 10-fold higher analgesic activity than (1S,2S)-(-)-tramadol (39).

Dosage for administration

After the rats were acclimated to the laboratory conditions, they were divided into groups depending on the dosage and time for sacrificing them. Five groups of six rats each were housed in separate cages. Tramadol was administered subcutaneously at 0, 24, and 48 h. The time periods chosen

for experiments following administration of the drug were 3, 6, 12, 24, 48, and 72 h. All doses were given in the morning between 9 and 10 h, keeping in view the altered activity of rats during the nights compared to the daytime. Controls were maintained individually for each group. In the present study, tramadol was administered according to ED₅₀ value obtained in rats at 31 mg/kg in hot plate test (40). Tramadol was also active in all pain models although at considerably higher doses (20–160 mg/kg, *s.c.*) (41).

Isolation of tissues

The present study was carried out on different areas of the brain, viz. cerebral cortex (CC), cerebellum (CB), pons-medulla (PM), hippocampus (HI) and thalamus (TH). The rats were sacrificed at the time periods mentioned above by cervical decapitation under light ether anesthesia. The brain was isolated immediately and placed on a chilled glass plate. The brain areas were separated and isolation of synaptosomes was done using conventional methods (42–44) and the synaptosomal fraction was stored at –80°C until further use.

Cholinesterases

Acetylcholinesterase (AChE, E.C. 3.1.1.7) activity in brain regions and erythrocytes, and pseudocholinesterase (butyrylcholinesterase: BuChE, E.C. 3.1.1.8) activity in plasma and erythrocytes were estimated by the method of Ellmann et al. (45).

Assay of blood cholinesterases

Blood was collected from rats using a heparinized syringe. To 200 µL of blood, 5 to 8 mL of 0.1 M sodium phosphate buffer (pH 7.4) was added. The contents were centrifuged at 2000 × g for 15 min. The supernatant was collected for estimation of plasma cholinesterase (PChE) activity, and the pellet was digested in a few drops of 0.1% saponin. The volume of this hemolysate was made up to 6 mL with 0.1 M sodium phosphate buffer (pH 7.4). This hemolysate was used to estimate the erythrocyte cholinesterase (EAChE) activity.

Assay of plasma and erythrocyte butyrylcholinesterase (E.C. 3.1.1.8) activity

The reaction mixture contained 270 µmoles of 0.1 M sodium phosphate buffer (pH 8.0), 10 µmoles of dithionitrobenzoate, 1.5 µmoles of butyrylthiocholine iodide as substrate and 300 µL of plasma as the enzyme source. The initial absorbance of the reaction mixture was recorded at 412 nm in a Hitachi U-2000 spectrophotometer prior to addition

of the substrate. The reaction was initiated by adding butyrylthiocholine iodide. After 15 min of incubation at room temperature, the yellow color developed was read at 412 nm. The enzyme activity was expressed as µmoles of butyrylthiocholine iodide hydrolyzed/mL of blood/h.

Assay of brain acetylcholinesterase (E.C. 3.1.1.7) activity

The composition of the reaction mixture was the same as that for butyrylcholinesterase activity, except that acetylthiocholine iodide was used as the substrate and brain tissue was the enzyme source. A hundred µL of 2% brain tissue homogenate was prepared in 0.25 M ice-cold sucrose solution and was used as the enzyme source. The enzyme activity was expressed as µmoles of acetylthiocholine iodide hydrolyzed/mg protein/h.

Assay of acetylcholine content

ACh content was estimated by the method of Hestrin as given by Augustinsson (46). The rat brain regions were weighed accurately. The tissues were placed in glass test tubes and exposed to heat in a boiling water bath for 10 min to terminate AChE activity completely and to release any bound ACh. To this, 1 mL of alkaline hydroxylamine hydrochloride solution was added, followed by 1 mL of 1:1 HCl solution. The contents were mixed thoroughly and centrifuged at 1000 × g for 5 min. To the supernatant, 0.5 mL of 0.37 M ferric chloride solution was added, and the brown color developed was read at 540 nm against a reagent blank. The acetylcholine content was expressed as µmoles of ACh/g wet weight of tissue.

Statistical analysis

All the assays were carried out with six separate replicates from each group. The mean and standard deviation (SD) were worked out using INSTAT statistical software, and analysis of variance (ANOVA) was done using SPSS statistical software using basic programming techniques on IBM compatible personal computer for different parameters. Difference between control and experimental assays was considered as not significant above $p < 0.05$.

RESULTS

Acetylcholine content

The ACh content in control rats was found to be the highest in the TH, followed by CC, HI, CB and PM [Table 1]. The content showed an increase in all areas of the brain, with the highest increase in

Table 1. Changes in acetylcholine (ACh) content in different brain regions of rat at different time periods after administration of tramadol at 0, 24, and 48 h.

Brain Area	Indices	Control	3 h	6 h	12 h	24 h	48 h	72 h
Cortex	Mean	2.78	4.27	4.07	3.76	3.17	3.29	3.38
	SD	± 0.31	± 0.34	± 0.34	± 0.35	± 0.41	± 0.44	± 0.21
	% change	—	+53.60*	+46.40*	+35.25*	+14.03	+18.34	+21.58
Cerebellum	Mean	1.31	1.76	1.69	1.50	1.24	1.39	1.45
	SD	± 0.11	± 0.13	± 0.17	± 0.15	± 0.14	± 0.22	± 0.21
	% change	—	+34.35*	+29.00*	+14.50	- 5.34	+6.11	+10.69
Pons-medulla	Mean	1.23	1.54	1.47	1.33	1.15	1.31	1.37
	SD	± 0.14	± 0.15	± 0.17	± 0.13	± 0.13	± 0.14	± 0.22
	% change	—	+25.20*	+19.51*	+8.13	- 6.51	+6.50	+11.38
Hippocampus	Mean	1.56	2.00	1.93	1.88	1.70	1.73	1.76
	SD	± 0.18	± 0.18	± 0.17	± 0.18	± 0.18	± 0.21	± 0.14
	% change	—	+28.20*	+23.72*	+20.51*	+8.97	+10.90	+12.82
Thalamus	Mean	3.24	4.58	4.72	3.41	3.08	3.52	3.69
	SD	± 0.12	± 0.19	± 0.20	± 0.17	± 0.15	± 0.12	± 0.18
	% change	—	+41.36*	+45.68*	+5.25	- 4.94	+8.64	+13.89

Each value is the mean ± standard deviation of observations from six separate experiments. Values are significant at least at $p < 0.05$ in SNK test. *Significant.

Table 2. Changes in acetylcholinesterase (AChE) activity levels in different brain regions of rats at different time periods after administration of tramadol at 0, 24 and 48 h.

Brain Area	Indices	Control	3 h	6 h	12 h	24 h	48 h	72 h
Cortex	Mean	3.86	2.36	2.68	2.87	3.54	4.19	4.45
	SD	± 0.86	± 0.70	± 0.61	± 0.68	± 0.67	± 0.88	± 0.26
	% change	—	- 38.86*	- 30.57*	- 25.65*	- 8.29	+8.55	+15.28
Cerebellum	Mean	3.44	2.08	2.24	2.50	2.97	1.39	1.45
	SD	± 0.57	± 0.24	± 0.32	± 0.37	± 0.45	± 0.22	± 0.21
	% change	—	- 39.54*	- 34.88*	- 27.33*	- 13.66	+6.11	+10.69
Pons-medulla	Mean	4.17	2.37	2.67	2.84	3.02	3.66	4.04
	SD	± 0.68	± 0.47	± 0.39	± 0.29	± 0.34	± 0.65	± 0.59
	% change	—	- 43.17*	- 35.97*	- 31.90*	- 27.58*	- 12.23	+3.12
Hippocampus	Mean	4.49	2.88	2.99	3.34	4.14	4.61	4.73
	SD	± 0.45	± 0.24	± 0.33	± 0.44	± 0.44	± 0.14	± 0.38
	% change	—	- 35.86*	- 33.41*	- 25.61*	- 7.80	+2.67	+5.34
Thalamus	Mean	4.26	2.45	2.64	2.98	3.58	4.48	3.65
	SD	± 0.78	± 0.35	± 0.39	± 0.43	± 0.38	± 0.12	± 0.41
	% change	—	- 42.49*	- 38.03*	- 30.05*	- 15.96	+5.16	+9.15

Each value is the mean ± standard deviation of observations from six separate experiments. Values are significant at least at $p < 0.05$ in SNK test. *Significant.

Table 3. Changes in the activity levels of plasma cholinesterases (PChE) and erythrocyte cholinesterases (EChE) in rats at different time periods after administration of tramadol at 0, 24 and 48 h.

Brain Area	Indices	Control	3 h	6 h	12 h	24 h	48 h	72 h
Plasma cholinesterase	Mean	24.92	36.11	40.18	28.37	25.80	27.12	28.58
	SD	± 2.81	± 2.21	± 2.86	± 2.32	± 2.60	± 2.63	± 2.27
	% change	—	+44.90*	+61.23*	+13.84	+3.53	+8.83	+14.68
Erythrocyte cholinesterase	Mean	111.32	131.06	135.14	125.37	113.68	116.87	121.21
	SD	± 3.52	± 4.10	± 3.91	± 3.10	± 2.84	± 2.06	± 3.68
	% change	—	+17.73*	+21.40*	+12.63*	+2.12	+4.98	+8.88

Each value is the mean ± standard deviation of observations from six separate experiments. Values are significant at least at $p < 0.05$ in SNK test. *Significant.

CC at 3 h following the administration of single dose of tramadol. The increase was also high in TH recorded at 3 and 6 h. Minimal elevation was recorded in PM, at 3 h after tramadol injection. The ACh levels were found elevated in all areas of the brain both at 48 and 72 h when tramadol was administered for the 2nd and 3rd time. The actual levels were higher in TH compared to CC, but the percent changes upon the administration of tramadol were higher in CC than in TH (Table 1). The changes at 48 and 72 h were quite perceptible but not statistically significant.

Acetylcholinesterase activity

The AChE activity in control rats was the highest in HI, followed by TH, PM, CC and CB (Table 2). Following the injection of tramadol, the AChE activity was found to decrease in all the brain areas, with quantitative differences between them. Maximal decrease was recorded in PM at 3 h, and minimal decrease was noticed in HI, again at 3 h. The decreases were intermediate in the remaining areas. However, the AChE activity showed a positive deviation in all areas of the brain at 48 and 72 h, when tramadol was administered for the 2nd and 3rd time. Higher levels were recorded in CC and TH compared to the other areas. The changes at 48 and 72 h were not statistically significant.

Plasma and erythrocyte cholinesterase activities

PChE and EChE showed far higher activity levels, with EChE showing a higher level of activity compared to PChE (Table 3). In both cases the enzyme activity showed an initial elevation at 3 h, which subsequently peaked at 6 h. PChE recorded higher elevation in activity compared to EChE.

Following this, along with ACh and AChE in brain areas, the PChE and EChE activities started reverting to the control levels, and by 24 h following tramadol dosage, both PChE and EChE activities reached the respective control levels. Following this, the activity levels of both PChE and EChE showed elevation over the control both at 48 and 72 h when tramadol was administered for the 2nd and 3rd time. The changes at 48 and 72 h were not statistically significant.

DISCUSSION

The basic idea behind carrying out his work was not to look at the pain mechanism or pain pathway. It was intended to examine the biochemical profile of the brain when an analgesic drug is administered when the animal is not subjected to any pain.

It would be interesting to see what an analgesic drug does to the system apart from subsiding pain. What an analgesic drug does in addition to anti-nociception is a matter of biological curiosity. Less emphasis has been laid on simpler biochemical parameters like enzymatic analyses, changes in amino acid levels and neurotransmitter levels in different areas of the brain, including the target and non-target ones. Further, simultaneous exploration of different transmitter systems and associated enzymes, besides other biochemical changes in different areas of the brain, has not been attempted. Despite the logical assumption that the actions of analgesic drugs would probably be the same in the presence or absence of pain, the biochemical implications of their actions in different areas of the brain remain relatively unexplored.

Tramadol is a narcotic-like pain reliever. It is a centrally acting synthetic analgesic of the aminocyclohexanol group with opioid-like effects. It is not derived from natural sources, nor is it chemically related to opiates. Anti-nociceptive drugs exercise their effects through neural pathways, and so choice of brain for the present study was a logical course. Tramadol crosses the placental and blood-brain barriers. Since the brain areas do not exist in isolated compartments but function as an integrated system, it is of interest to examine what would be the responses in different other areas of the brain when a drug with specific target area(s) is administered. Hence, an attempt was made to examine the biochemical profiles in different areas of rat brain, both target and non-target, in the sense with pain centers like thalamus and cortex, during the administration of an analgesic drug, taking the cholinergic segment as an index.

In the current study, the possible effect of tramadol was reviewed from aspects of its effects on the cholinesterase activity levels during no-pain condition. Analgesic activity and central antinociceptive action of cholinergic and cholinomimetic agents such as atropine, methylatropine, mecamlamine, physostigmine, neostigmine and carbachol were examined (47–49), and it was proposed that ACh and carbachol are capable of inducing an antinociceptive effect through stimulation of the central muscarinic receptors.

Previous reports have indicated the presence of ACh, AChE, and CAT in many regions of the CNS (50–53). In the present work, the highest AChE activity was recorded in HI, although the highest ACh content was found in TH. Following subcutaneous administration of single dose of tramadol, the cholinergic parameters showed differential quantita-

tive changes, both with reference to each parameter as well as the area of brain even during non-induction of pain. Maihöfner (54) suggested that there is a difference in the brain areas modulated by analgesia and antihyperalgesia. The depression of cholinergic transmission in the CNS as a result of opioid-induced inhibition of acetylcholine release may be an important mechanism for the analgesic and side-effects of opioids.

Opioids upon binding with the receptors inhibit the release of excitatory neurotransmitters from the axon terminals of neurons carrying nociceptive stimuli (55). The content of ACh showed an increase following the injection of tramadol, with the peak increase occurring at 3 or 6 h after tramadol injection in the present study.

The ACh content, and AChE and pseudo-cholinesterase activities recorded changes that were maximal at 3 or 6 h following tramadol administration. After this, the ACh content and cholinesterase activities started returning to the control levels during the subsequent periods, reaching more or less the control levels by 24 h after administration. However, the ACh content in the cerebral cortex was still higher than the control by 24 h, and the AChE activity was still much lower than the control in cerebellum, pons-medulla and thalamus, which indicated a prolonged effect.

With reference to the dosage of tramadol used in the present study, for treatment of pain in humans, tramadol is recommended in a dose of 50–100 mg orally every 4 h (56). In animal experiments, it has been reported that CNS reactions such as convulsion, tremor, ataxia, salivation, mydriasis and dyspnea were seen with very high oral or subcutaneous doses of tramadol of several hundred mg/kg (57). Tramadol (20 mg/kg, *i.p.*) administered acutely (single dose) in male Wistar rats, induced a significant decrease in the α_2 -adrenergic receptors at 24 h after dosing in all brain regions studied. The most pronounced effects were observed in all sub-regions of the olfactory system, nucleus accumbens and septum, thalamus, amygdala, and cerebral cortex (58), a finding that agrees with the greater effect on cerebral cortex and thalamus in the present study. In the present study, the changes in cholinergic parameters were followed at shorter time intervals up to 24 h after the administration of a single dose of tramadol. Following this, the 2nd and 3rd doses were given at 24 and 48 h and the changes followed at 48 and 72 h.

After oral administration, tramadol was found to show 68% bioavailability, with peak serum concentrations reached within 2 h, and the half-life of

tramadol being 5.1 h. As has been shown by Matthiesen et al. (59), the terminal elimination half-life of tramadol in the rat is about 3 h. After repeated administration, there is no evidence of accumulation of the compound or increased metabolism (60, 61). It has been demonstrated that 1 h after a single oral dose, tramadol concentrations in the plasma of majority of patients are sufficient to give good pain reduction (60). While morphine remains one of the most widely used opioids for the treatment of painful conditions, other opioids are also commonly employed. In rats studied 4 h after treatment, all the opioids except tramadol (10 mg/kg) decreased plasma testosterone in comparison with saline administration. Twenty-four hours after treatment, plasma testosterone levels were different (higher) than control in the animals treated with the low doses of morphine, fentanyl and buprenorphine. Four hours after treatment, brain testosterone was drastically decreased in all groups except buprenorphine, in which it remained at control levels. All groups returned to control levels at 24 h after treatment.

The duration of analgesic effect of a single oral dose of 100 mg of tramadol has been reported to be 6 h (62). Thus, the effect of subcutaneous dose (31 mg/kg) used in the present study appears to be matching with that of the single oral dose reported by Dayer et al. (62). A low abuse liability is reported for tramadol, centrally acting through either opioid or nonopioid mechanisms. The effects of the repeated administration of different doses of tramadol (10, 20, and 80 mg/kg, *i.p.*) were evaluated on the opioid precursor prodynorphin biosynthesis (63) in comparison with morphine (10 mg/kg, *i.p.*), in the rat CNS, tramadol did not cause any significant change in the striatum, and did not decrease prodynorphin biosynthesis in the hypothalamus and in the hippocampus, at nontoxic doses (10 and 20 mg/kg). The above results agree with our study design, as tramadol was given at 30 mg/kg body weight chronically for 3 days.

In the light of this, the maximal positive or negative peaks at 3 or 6 h observed for the cholinergic parameters examined in this study appear logical. Further, in treatment schedules for humans in pain tramadol is recommended in a dose of 50–100 mg orally every 4 h. The prolonged elevation or decrease observed for ACh and AChE, respectively, at 24 h after tramadol administration in certain areas may be due to secondary effects caused by various other factors (Tables 1, 2). The changes in parameters related to the cholinergic system suggest that tramadol may exercise its effects through changes in ACh levels as one of the facets of its analgesia. The

ACh levels are probably regulated by the activity of AChE. The pseudocholinesterase activities presumably get geared up through elevations to aid in energy-related functions.

The ACh content continued to show positive deviations in all areas of the brain at 48 and 72 h when tramadol was administered for the 2nd and 3rd time, i.e., at 24 and 48 h, respectively (Table 1). This indicates prolonged action of the drug upon repeated dosing. However, the elevations at 48 and 72 h were not statistically significant compared to those during different periods of the first 24 h after the initial single dose. This is attributable to differential intensities in tissue responses to single and multiple dosing, the basic response remaining the same. This augurs well for the use of tramadol as an analgesic over longer periods. Further, it is also interesting that the AChE activity, which showed a decrease in all areas of the brain after single dose of tramadol, tended towards positive deviations in all areas after 2nd and 3rd doses, at 48 and 72 h (Table 2). Presumably, the increase in activity of AChE at 48 and 72 h could be a means of keeping a check on the increase in ACh content during these periods following multiple dosing with tramadol. Such compensatory physiological homeostatic mechanisms were implicated earlier with reference to the action of the organophosphate diisopropylfluorophosphate on the cholinergic segment in swine tracheal smooth muscle (64, 65).

The present study demonstrates that administration of tramadol causes considerable changes in the levels of ACh content, AChE and pseudocholinesterase activities without any reference to the induction of pain. The observed changes in the cholinergic segment presumably do not cause any physiological lesion since they reverted to control levels or possibly homeostatically controlled after the time limit of change under tramadol influence. This observation indicates that tramadol can be administered safely both under nociceptive and non-nociceptive conditions.

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