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Effect of paracetamol on central 5-HT_{2A} serotonin receptors

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Tarasub N, Srikiatkachorn A, Govitrapong P. Effect of paracetamol on central 5-HT_{2A} serotonin receptors. *Chula Med J* 1997 Dec; 41 (12): 877-88

Background : *Precise mechanisms underlying the antinociceptive effect of paracetamol are not fully understood. Recent evidence indicates that a central mechanism, possibly serotonin dependent, may be involved in the process.*

Objective : *To study the acute and chronic effects of paracetamol on the binding characteristics of 5-HT_{2A} serotonin receptors in different regions of rat brains.*

Setting : *Department of Physiology, Faculty of Medicine, Chulalongkorn University and the Neuro- and Behavioural Biology Center, Institute of Science and Technology for Research and Development, Mahidol University.*

Research design : *Animal experiment.*

Materials : *The studied animals comprised 4 groups of adult Wistar rats, i.e. acute, 15 days-paracetamol treated, 30 days-paracetamol treated and control groups. The paracetamol treated groups were further divided into subgroups receiving various dosages of paracetamol.*

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Methods : *Paracetamol was administered intraperitoneally for the assigned period. After receiving the full course of drug administration, the animals were sacrificed and cerebral cortex and brainstem were isolated. Characteristics of specific binding of 5-HT_{2A} serotonin receptors on cortical and brainstem tissues were determined using a radioligand binding method.*

Results : *A significant reduction of maximum receptor number (B_{max}) was observed in cortical tissue of acutely paracetamol treated groups (B_{max} values = 1.14 ± 0.08 , 1.20 ± 0.15 and 2.13 ± 0.09 pmol/mg protein, for 300 and 400 mg/kg paracetamol treated and control groups, respectively, $p < 0.001$), whilst the dissociation equilibrium constant (K_d) remained unchanged. No significant difference was noted when values of B_{max} and K_d of 5-HT_{2A} receptor binding on brainstem tissue were compared. Down-regulation of cortical 5-HT_{2A} receptors was more prominent in animals receiving paracetamol for 15 days, especially in animals treated with higher doses of paracetamol. The B_{max} value of 5-HT_{2A} receptor binding on cortical tissue decreased after treating with paracetamol for 30 days.*

Conclusions : *Our findings showed that administration of paracetamol can alter central 5-HT_{2A} receptors. This data implies the possible involvement of the 5-HT system in antinociceptive efficacy of non-narcotic analgesics and therefore further supports the central hypothesis of an antinociceptive mechanism of these agents.*

Key words : *Paracetamol, Serotonin, Serotonin receptor, Analgesics.*

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- เหตุผลของการวิจัย** : กลไกการออกฤทธิ์แก้ปวดของยาพาราเซตามอลยังไม่เป็นที่ทราบแน่ชัด หลักฐานในปัจจุบันบ่งชี้ว่าอาจเกี่ยวข้องกับการทำงานของระบบประสาทส่วนกลาง โดยเฉพาะที่สัมพันธ์กับระบบซีโรโตนิน
- วัตถุประสงค์** : เพื่อศึกษาผลของการบริหารยาพาราเซตามอลอย่างเฉียบพลันและเรื้อรังต่อลักษณะการเกาะจับของตัวรับซีโรโตนินในส่วนต่างๆ ของสมองหนู
- สถานที่ที่ทำการศึกษา** : ห้องปฏิบัติการภาควิชาสรีรวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย และโครงการวิจัยชีววิทยาระบบประสาทและพฤติกรรมกรรมสถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยมหิดล
- รูปแบบการวิจัย** : การทดลองในสัตว์ทดลอง
- สัตว์ทดลอง** : ทำการศึกษาในหนูพันธุ์ Wistar ซึ่งแบ่งออกเป็น 4 กลุ่ม ได้แก่ กลุ่มที่ได้รับยาพาราเซตามอลอย่างเฉียบพลัน, ได้รับยานาน 15 วัน, 30 วัน และกลุ่มควบคุมโดยแบ่งกลุ่มที่ได้รับยาพาราเซตามอลออกเป็นกลุ่มย่อยเพื่อศึกษาผลที่เกิดขึ้นจากการได้รับยานานต่างๆ
- วิธีการศึกษา** : ทำการศึกษาโดยบริหารยาพาราเซตามอลเข้าช่องท้องของหนูตามระยะเวลาที่กำหนด จากนั้นทำให้หนูเสียชีวิต ตัดแยกเนื้อเยื่อสมองบริเวณกลีบพอนทาลของสมองใหญ่และก้านสมอง ทำการศึกษาลักษณะการเกาะจับของตัวรับซีโรโตนินชนิด 5-HT_{2A} ในเนื้อเยื่อสมองโดยใช้เทคนิคการเกาะจับของสารรังสี
- ผลการศึกษา** : การบริหารยาพาราเซตามอลอย่างเฉียบพลันทำให้เกิดการลดต่ำลงของความหนาแน่นของตัวรับในสมองใหญ่ส่วนพอนทาลอย่างมีนัยสำคัญทางสถิติ (ค่า B_{max} = 1.14 ± 0.08 , 1.20 ± 0.15 and 2.13 ± 0.09 pmol/mg protein, สำหรับกลุ่มที่ได้รับยาพาราเซตามอล 300 และ 400 มิลลิกรัม/กิโลกรัม และกลุ่มควบคุมตามลำดับ, $p < 0.001$) โดยไม่พบการเปลี่ยนแปลงของค่าคงที่ของสมดุลการแยกตัว (K_d) สำหรับลักษณะการเกาะจับของตัวรับ 5-HT_{2A} ในก้านสมองไม่มีการเปลี่ยนแปลงการลดลงของตัวรับจะเห็นชัดเจนยิ่งขึ้นในหนูที่ได้รับยาพาราเซตามอลเป็นเวลา 15 วัน และความหนาแน่นของตัวรับมีการปรับตัวสูงขึ้นในหนูที่ได้รับยาพาราเซตามอลเป็นเวลา 30 วัน
- สรุป** : ผลการศึกษาแสดงให้เห็นว่ายากแก้ปวดพาราเซตามอลสามารถทำให้เกิดการเปลี่ยนแปลงของระบบตัวรับ 5-HT_{2A} ในระบบประสาทส่วนกลางซึ่งบ่งชี้ถึงบทบาทของระบบซีโรโตนินในกระบวนการออกฤทธิ์บรรเทาอาการปวดของยาแก้ปวดประเภทไม่เสพติด

Despite their wide usage, pharmacological mechanisms underlying the antinociceptive effects of non-narcotic analgesics are still an issue of controversy. Conventionally, it is believed that analgesia produced by non-narcotic analgesics results from suppression of prostaglandin synthesis due to cyclo-oxygenase inhibition. However, although the anti-inflammatory potency of these agents are well correlated to their inhibition of cyclo-oxygenase, this correlation cannot be observed between antinociceptive potency and the activity of enzyme inhibition.

While several lines of evidence contradict the peripheral action of analgesics, accumulating data imply that these drugs may exert their antinociceptive effect via a centrally mediated mechanism. In 1992, Björkman, et al, demonstrated that local injection of diclofenac into rat nucleus raphe magnus, a major serotonergic cell group, produced a more pronounced antinociceptive effect as compared to subcutaneous, intrathecal or intracerebroventricular injection.⁽¹⁾ It has long been known that serotonin (5-hydroxytryptamine: 5-HT) plays a pivotal role in pain modulation.⁽²⁾ Release of 5-HT from raphe nuclei neurons that terminate in the spinal dorsal horn is an important step in the endogenous pain control mechanism. Administration of morphine, either systemic, intraventricular or direct injection into periaquiductal gray matter, evoked the release of 5-HT from spinal cord terminals.^(3,4) A significant increase in tissue levels of 5-HT was observed in cat spinal dorsal horn after electrical stimulation of

the dorsal column.⁽⁵⁾ Roles of 5-HT in the antinociceptive effect of non-narcotic analgesics have been suggested.⁽⁶⁾

Paracetamol (acetaminophen) has been extensively used for the treatment of pain and fever. As with other non-narcotic analgesics, the mechanism of the therapeutic action of paracetamol is still not known and it is unclear whether it acts peripherally, centrally or both. Since paracetamol possesses insignificant peripheral anti-inflammatory effects as compared to other non-steroidal anti-inflammatory drugs (NSAIDs), a central mechanism of action has been postulated. Various physiologic mechanisms have been proposed for paracetamol-induced analgesia, i.e. central inhibition of prostaglandin synthesis, modulation of the central L-arginine-nitric oxide pathway,⁽⁷⁾ etc. As a central 5-HT dependent antinociceptive system seems to be a "common pathway" of mechanisms underlying antinociceptive efficacy of several analgesics, its role in paracetamol-induced analgesia is possible.

A relationship between the 5-HT system and paracetamol has been demonstrated previously. Acute administration of paracetamol increased 5-HT levels in cerebral cortex and pons in experimental animals.⁽⁸⁾ Chronic use of paracetamol can deplete 5-HT from platelets and eventually up-regulate the 5-HT_{2A} receptors on platelet membranes in migraine patients.^(9,10) The present study was conducted to further investigate the effects of paracetamol on central 5-HT_{2A} receptors as well as the plasticity of this receptor system after chronic paracetamol exposure.

Materials and Methods:

Animals

Adult male Wistar rats weighing 240–360 g at the beginning of the experiments were used in this study. The experimental animals were supplied by the National Laboratory Animal Center of Mahidol University, and were maintained on normal rat food and tap water *ad libitum* under controlled environmental conditions.

Drug Administration

The rats were divided into several experimental groups of 4–6 animals each. To study the acute effects of paracetamol administration, the rats were injected intraperitoneally with paracetamol, dissolved in a vehicle which consisted of 12.5% propylene glycol in normal saline. Dosage of 300 and 400 mg/kg/d paracetamol in a volume of 10 ml/kg were used in this experiment. In the chronic paracetamol treated group, various dosages of paracetamol (200, 300 and 400 mg/kg/day) were given intraperitoneally every day for durations of 15 and 30 days. In all experiments an equal volume of normal saline was used as control for injections and the same procedure was followed as for the treated groups. To minimize the influence of circadian rhythms, all groups of rats were treated at the same time of the day.

One day after receiving the full course of drug administration, the rats were sacrificed by decapitation. The brains were rapidly removed and were dissected. Two regions of the brain, the frontal cortex and the whole brain stem, were isolated and kept at -80°C until assayed.

Membrane Preparation

The dissected brain tissues were weighed and 20 volumes of ice-cold 50 mM Tris HCl buffer (pH 7.4) was added and then homogenized for 15 seconds with a tissue homogenizer set at 13,500 rpm. The homogenate obtained was centrifuged at 1,000 g for 5 minutes. The supernatant was further centrifuged at 40,000 g for 20 minutes at 4°C in a refrigerated centrifuge (Dupont, Sorvall RC 26 plus). The supernatant was decanted and the membrane pellet was resuspended in 20 volumes of ice cold 50 mM Tris HCl with pH 7.5 and homogenized. The process was repeated twice with 50 mM Tris HCl for washing the membrane pellet. The membrane pellet was then resuspended into the incubation buffer (containing 120 mM NaCl, 5 mM KCl, 1 mM MgCl_2 and 2 mM CaCl_2 in 50 mM Tris HCl buffer, pH 7.5) to form the final membrane suspension for the binding studies.

Method Radioligand Binding Assay

The freshly prepared membrane was resuspended in 20 volumes of ice cold 50 mM Tris HCl salt buffer (pH 7.5) containing 120 mM NaCl, 5 mM KCl, 1 mM MgCl_2 and 2 mM CaCl_2 and homogenized for 15 seconds with a tissue homogenizer set at 13,500 rpm. All binding assays were carried out by using sufficient membrane preparation to provide a tissue protein concentration between 0.1–0.3 mg/ml. Assays were performed by the addition of 400 μl of membrane suspension into glass tubes containing 50 μl incubation buffer with or without appropriate drug in buffer. Six to ten concentrations varying

from 0.4-12 nM of (phenyl-4 ^3H)-spiperone (Amersham, UK) were added to the tissue suspension making a final incubation volume of 500 μl . The assay mixture was incubated at 37°C for 30 minutes, during which time equilibrium was reached. The reaction was then terminated by rapid filtration through a glass microfiber filter (Whatman GF/C, Whatman International Ltd., Maidstone, UK) under vacuum. The filters were washed twice with ice cold Tris buffer. Receptor-bound radioactivity was counted in volumes of 5 ml scintillation fluid containing Triton X 100/toluene base fluor (1:3) by a scintillation counter (Beckman LS 1801). Specific (^3H)-spiperone binding, which was defined as the excess over blanks taken in the presence of 10 μM of ketanserin (Janssen Research Foundation, Belgium), accounted for 40-60% of the total binding. All experiments were performed in duplicate. The protein concentrations of the membranes were estimated by Lowry's method using bovine serum albumin as a standard.

Data Analysis

The saturation curve was analyzed by the Scatchard method and then applied to the LIGAND non-linear least regression analysis computer program, for analyzing the relationship between the bound/free versus bound fraction. The data were expressed in a dissociation equilibrium constant (K_d) and maximum number of receptor sites (B_{max}) as mean \pm SEM. Statistical evaluation of the results was performed using mixed analysis of variance (ANOVA) and Student's t-test.

Results

Effect of Acute Paracetamol Administration on Central 5-HT_{2A} Receptors

The results revealed B_{max} values of 5-HT_{2A} receptors on the cortical membrane for 300 and 400 mg/kg paracetamol-treated groups and control to be 1.14 \pm 0.08, 1.20 \pm 0.15 and 2.13 \pm 0.09 pmol/mg protein, respectively. The difference between the values of paracetamol-treated groups and control were statistically significant ($p < 0.001$). No significant difference was evidenced when B_{max} values of 5-HT_{2A} receptor binding on brainstem tissue were compared (1.01 \pm 0.08, 1.07 \pm 0.05 and 1.10 \pm 0.16 pmol/mg protein, for 300 and 400 mg/kg paracetamol-treated and control groups, respectively). The values of K_d were comparable in all groups (Table 1).

Effect of Chronic Paracetamol Administration on Central 5-HT_{2A} Receptors

Our results showed that the B_{max} values of 5-HT_{2A} receptors on the cortical membrane of 300 and 400 mg/kg/d paracetamol-treated groups for 15 days and control were 1.34 \pm 0.11, 0.94 \pm 0.10 and 2.18 \pm 0.15 pmol/mg protein, respectively. The difference between the values of paracetamol-treated groups and control were statistically significant ($p < 0.001$). This receptor down-regulation seemed to be dose-dependent, as no such change was observed in rats daily-treated with 200 mg of paracetamol (B_{max} value = 1.93 \pm 0.31 pmol/mg protein). The values of K_d remained unchanged in all groups. None of paracetamol doses used was able to modify either

Table 1. Effects of paracetamol treatment on [³H]-spiperone bindings on neural membrane. Significant difference was observed (* p<0.001) when B_{max} values of 5-HT_{2A} receptors on cortical tissues of paracetamol treated groups and controls were compared.

Group	Frontal Cortex			Brainstem	
	n.	B _{max} (pmol/mg protein)	K _d (nM)	B _{max} (pmol/mg protein)	K _d (nM)
Control	6	2.13±0.09	1.36±0.05	1.10±0.16	0.93±0.09
Paracetamol					
300 mg/kg/day	5	1.14±0.08*	1.20±0.32	1.01±0.08	0.92±0.12
400 mg/kg/day	5	1.20±0.11*	1.06±0.23	1.07±0.05	0.88±0.07

B_{max} or K_d of 5-HT_{2A} receptors in the brainstem. for 200, 300 and 400 mg/kg/d paracetamol-
(B_{max} values = 1.02±0.08, 1.14±0.29, 1.27±0.23 and treated groups and control group, respectively)
1.05±0.14 pmol/mg protein and K_d values = (Table 2).
0.83±0.13, 0.87±0.24, 1.00±0.11 and 0.88±0.14 nM

Table 2. Effects of paracetamol administration on 5-HT_{2A} receptor in rat brain. Significant difference was observed (* p<0.01, ** p<0.001) when B_{max} values of 5-HT_{2A} receptors on frontal tissues of 15-day, 30-day paracetamol treated groups and controls were compared.

Group	Frontal Cortex			Brainstem	
	n.	B _{max} (pmol/mg protein)	K _d (nM)	B _{max} (pmol/mg protein)	K _d (nM)
Control	6	2.18±0.15	1.43±0.15	1.05±0.14	0.88±0.14
15 day paracetamol					
200 mg/kg/day	4	1.93±0.31	1.57±0.34	1.02±0.08	0.83±0.13
300 mg/kg/day	5	1.34±0.11*	1.08±0.08	1.14±0.29	0.87±0.24
400 mg/kg/day	5	0.94±0.10**	1.24±0.10	1.27±0.23	1.00±0.11
30 day paracetamol					
300 mg/kg/day	5	1.38±0.12*	1.22±0.11	0.92±0.12	0.70±0.11
400 mg/kg/day	5	1.34±0.13*	1.12±0.19	0.82±0.12	0.82±0.08

Decreased degrees of paracetamol-induced 5-HT_{2A} receptor down-regulation were observed in the cortical tissue of rats treated with paracetamol for 30 days (B_{\max} values = 1.38±0.12, 1.34±0.13 and 2.05±0.20 pmol/mg protein, for 300 and 400 mg/kg/d paracetamol treated and control groups, respectively). This observation was more prominent in animals receiving higher doses (400 mg/kg/d) of paracetamol. The difference between B_{\max} values of 5-HT_{2A} receptor binding on cortical membrane of 400 mg paracetamol treated groups for the periods of 15 and 30 days was statistically significant ($p < 0.01$). No significant difference was observed when mean values of K_d were compared. All of the saturation parameters of 5-HT_{2A} receptor binding on brainstem tissue were unchanged.

Discussion

The results of this study demonstrated that administration of paracetamol can alter the central 5-HT system by down-regulating the 5-HT_{2A} receptors in the frontal cortex. Such receptor down-regulation was observed in both acute and 15-day paracetamol treated groups. The acute effect of paracetamol on 5-HT_{2A} receptors reported herein corresponds well with an observation of Pini, et al.⁽⁸⁾ Interestingly, the degree of receptor-down regulation became less evidenced after administration of paracetamol for 30 days. This paracetamol-induced change of B_{\max} values implies an involvement of the 5-HT system in the antinociceptive activity of paracetamol.

Serotonin is considered an extremely significant neurotransmitter in the endogenous pain control mechanism. Recent evidence showed that the antinociceptive effect of various analgesics, either narcotic or non-narcotic, depends on the integrity of the central 5-HT system. For example, intrathecal injection of 5-HT antagonist can attenuate the analgesia produced by microinjection of morphine into periaquiductal gray matter.⁽¹¹⁾ Administration of *p*-chloro-phenylalanine, a tryptophan hydroxylase inhibitor, profoundly interferes with the antinociceptive effect of NSAIDs such as diclofenac, as well as paracetamol.^(6,8) In humans, a central analgesic effect after intravenously administering paracetamol was previously demonstrated using transcutaneous electrical stimulation of the sural nerve in healthy volunteers.⁽¹²⁾

Serotonin exerts its various physiological effects via a vast diversity of receptor subtypes.⁽¹³⁾ Regarding nociception, recent advances in the field of 5-HT receptor pharmacology show that the different 5-HT receptor subtypes play different roles. Several lines of evidence suggest that stimulation of 5-HT_{1A} receptors can elicit analgesia, whereas stimulation of 5-HT_{2A} receptors potentiate nociceptive transmission.⁽¹⁴⁾ The nociceptive facilitating activity of 5-HT₂ receptors possibly results from enhancing the release of algogenic peptides from primary afferents.⁽¹⁵⁾ A supraspinal mechanism of this 5-HT₂-induced nociceptive facilitation has also been suggested.⁽¹⁶⁾

Taking the above conclusion of a nociceptive facilitating effect of 5-HT_{2A} receptors, we suggest that the reduction of these receptors observed in this experiment might explain an antinociceptive efficacy of paracetamol.

Patterns of 5-HT receptor distribution in the central nervous system are complex and depend on the subtypes of the receptor. 5-HT₂ receptors reveal a specific distribution in many brain areas, including the frontal cortex.⁽¹⁷⁾ Actually, cerebral cortex presents highest densities of 5-HT₂ receptors. The presence of very high densities of these receptors in the neocortex, especially over the pyramidal cell layers which receive afferents from several central structures, suggests their involvement in the regulation of many brain functions, including nociceptive modulation. The reduction of the number of 5-HT_{2A} receptors in the cortical area, but not in the brainstem, could depend on the different density of receptors in these areas and would emphasize the role of the cortex as the target for the serotonergic antinociceptive system.

Mechanisms by which paracetamol induces 5-HT_{2A} receptor plasticity are not fully understood. It has recently been shown that paracetamol has no binding affinity to either types of 5-HT receptors or transporter.⁽¹⁸⁾ Since there is no evidence that paracetamol acts directly on any subtypes of 5-HT receptors, the plasticity of such receptors should result from other mechanisms. It has been shown that acute administration of paracetamol can increase the level of 5-HT in the cerebral cortex and pons.^(8, 19) Since changes in

concentration of neurotransmitters has a significant impact on receptor adaptation, a persistent exposure of agonists or endogenous neurotransmitters results in receptor down-regulation. Darmani and co-workers showed that after agonist exposure the ability of the 5-HT₂ receptor system to induce down-regulation appears in a relatively short period of time.⁽²⁰⁾ Therefore, we may assume that paracetamol, by indirectly increasing the concentration of the 5-HT, may induce an adaptive down-regulation of the post-synaptic 5-HT_{2A} receptors.

The temporal pattern of paracetamol-induced 5-HT_{2A} receptor adaptation observed in this study is of interest. Compared to other groups, the receptor down-regulation was best observed in the 15-day paracetamol administered group. In contrast, the receptor density became greater in rats receiving paracetamol for 30 days. Loss of analgesic efficacy has been previously observed in animals chronically treated with acetyl salicylate or phenaxone.⁽²²⁾ As analgesic efficacy seems to depend on 5-HT_{2A} receptor regulation, the reverse of such down-regulation observed in this group may relate to the loss of analgesic efficacy after chronic use. Moreover, it has been accepted that prolonged reductions of pain sensation by any process, including chronic analgesic consumption, often leads to functional changes that tend to restore sensitivity. The phenomenon of analgesic rebound headache⁽²¹⁾ may represent a situation where such a compensatory mechanism plays a major role in perpetuation of the pain. An up-

regulation of 5-HT_{2A} receptors has been reported in migraine patients with analgesic rebound headaches.⁽²³⁾ Since this subtype of 5-HT receptors is involved in pain facilitation, an increase in receptor numbers observed in these patients may result in a hyperalgesic state and the development of chronic daily headache.

Based on this assumption of paracetamol-induced 5-HT release, chronic use of this drug may deplete 5-HT from its storage sites. Indeed, depletion of platelet 5-HT content was demonstrated in patients with analgesic rebound headache.⁽⁹⁾ Chronic 5-HT depletion will consequently up-regulate the 5-HT_{2A} receptors. This hypothesis corresponds to the previous finding of an increase in 5-HT receptor numbers in rat cortical and pontine membranes after being chronically treated with the pyrazole derivative, phenazone.⁽²²⁾

In conclusion, this data provides further evidence for a central 5-HT dependent antinociceptive effect of paracetamol. Down-regulation of 5-HT_{2A} receptors in response to 5-HT release is a major step in understanding the mechanism underlying analgesia produced by this agent. On the contrary, chronic use of paracetamol may result in 5-HT depletion which in turn produces re-adaptation of the 5-HT_{2A} receptors. This receptor plasticity may be an important mechanism related to the loss of analgesic efficacy, and in the more extreme condition may produce analgesic-related painful conditions, e.g. analgesic rebound headaches, etc.

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