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**EFFECTS OF CHRONIC PARACETAMOL ADMINISTRATION
ON SEROTONERGIC NEUROTRANSMISSION**



MRS. NAOVARAT TARASUB

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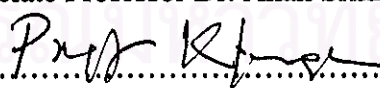
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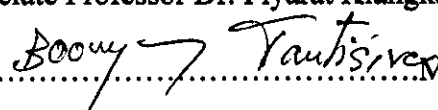

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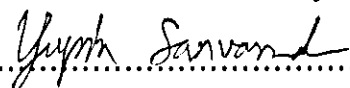
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พาราเซตามอล เป็นยาแก้ปวดที่นิยมใช้มากที่สุด กลไกการออกฤทธิ์แก้ปวดยังไม่ทราบแน่ชัด เพียงคาดว่า อาจเกี่ยวข้องกับระบบประสาทเซโรโทนิน ในการทดลองนี้ ได้ศึกษาผลของการได้รับยาพาราเซตามอลแบบเฉียบพลัน และ เรื้อรัง ผลการทดลอง พบว่า ในหนูกลุ่มที่ได้รับยาชนิดนี้ทางช่องท้อง 400 มิลลิกรัม/กิโลกรัม ทุกวัน เป็นเวลา 15 วัน และ แบบเฉียบพลัน มีความทนต่อความเจ็บปวดเพิ่มสูงขึ้น อย่างมีนัยสำคัญทางสถิติ ส่วนหนูที่ได้รับยาแบบเดียวกัน เป็น เวลา 30 วัน ไม่พบการเปลี่ยนแปลงชนิดนี้ เมื่อใช้เกร็ดเลือดเป็นตัวแทนในการศึกษาการเปลี่ยนแปลงของระบบประสาทเซโรโทนิน พบว่า ในหนูกลุ่มที่ได้รับยา 300 และ 400 มิลลิกรัม/กิโลกรัม ทุกวัน เป็นเวลา 15 วัน มีระดับเซโรโทนิน ในเกร็ด เลือดเพิ่มสูงขึ้น อย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มควบคุม (7024.67 ± 905.97 , 7342.83 ± 1041.35 และ 3911.32 ± 438.07 ng/ 10^5 platelets, $p < 0.01$) ส่วนหนูกลุ่มที่ได้รับยานาน 30 วัน ในขนาดเดียวกัน มีระดับเมตาบอไลต์ของเซโรโทนิน (5-HIAA) เพิ่มสูงขึ้นอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มควบคุม (13788.65 ± 2373.95 , 13816.77 ± 2517.06 และ 7116.84 ± 1199.23 ng/ 10^5 platelets, $p < 0.01$) การศึกษาผลการเปลี่ยนแปลงค่าความหนาแน่นสูงสุด (B_{max}) และ ค่าคงที่สมมูลของการแยก (K_d) ของตัวรับ ชนิด 5-HT_{2A} receptor และ ตัวเก็บกลับเซโรโทนิน (5-HT uptake sites) ในสมอง ส่วน frontal cortex และ brainstem โดยวิธีการจับติดของสารรังสี (radioli-gand binding technique) พบว่า ค่า B_{max} ของ 5-HT_{2A} receptor ลดลง อย่างมีนัยสำคัญทางสถิติในสมองส่วน frontal cortex ของหนูทุกกลุ่มที่ได้รับยา 300 และ 400 มิลลิกรัม/กิโลกรัม แต่ค่า K_d ไม่มีความแตกต่างทางสถิติ และ ในสมองส่วน brainstem ไม่พบการเปลี่ยนแปลงนี้ ผลการทดลอง ในกลุ่มที่ได้รับยานาน 30 วัน มีการปรับตัวเพิ่มค่า B_{max} ของ 5-HT_{2A} receptor สูงขึ้น เมื่อเปรียบเทียบกับกลุ่มที่ได้รับยาเป็นเวลา 15 วัน ในขนาด 400 มิลลิกรัม/ กิโลกรัม (0.94 ± 0.01 และ 1.34 ± 0.13 pmol/mg protein ตามลำดับ)

การเปลี่ยนแปลงค่า B_{max} ของตัวเก็บกลับเซโรโทนิน ได้ผลตรงกันข้ามกับที่พบในตัวรับ 5-HT_{2A} receptor ผลการทดลอง พบว่าที่สมองส่วน frontal cortex ในหนูทุกกลุ่มที่ได้รับยา 300 และ 400 มิลลิกรัม / กิโลกรัม มีค่า B_{max} ของ ตัวเก็บกลับเซโรโทนิน เพิ่มสูงขึ้น อย่างมีนัยสำคัญทางสถิติ แต่ค่า K_d ไม่มีความแตกต่างทางสถิติ และ ในสมองส่วน brainstem ไม่พบการเปลี่ยนแปลงนี้ ผลการทดลอง ในกลุ่มที่ได้รับยานาน 30 วัน มี ค่า B_{max} ของ ตัวเก็บกลับเซโรโทนิน ลดลง เมื่อเปรียบเทียบกับกลุ่มที่ได้รับยาเป็นเวลา 15 วัน ในขนาด 400 มิลลิกรัม/ กิโลกรัม (4.59 ± 0.52 และ 2.71 ± 0.18 pmol/mg protein ตามลำดับ) จากผลการทดลองนี้ แสดงให้เห็นว่า มีการปรับตัวลดค่า B_{max} ของตัวรับ ชนิด 5-HT_{2A} receptor ที่ postsynaptic membrane และ เพิ่ม ค่า B_{max} ของตัวเก็บกลับเซโรโทนิน ที่ สมองส่วน frontal cortex ในกลุ่มที่ได้รับยาทุกวัน เป็นเวลา 15 วัน และแบบเฉียบพลัน ซึ่งการเปลี่ยนแปลงนี้จะเกิดขึ้นพร้อมๆ กับการเพิ่มสูงขึ้นของระดับเซโรโทนิน และ ความทนต่อความเจ็บปวด การเปลี่ยนแปลงของตัวรับ 5-HT_{2A} receptor และ ตัวเก็บกลับเซโรโทนิน ที่เกิดขึ้นเมื่อได้รับยาเป็นเวลา 15 วัน จะกลับคืนสู่สภาวะปกติได้ เมื่อได้รับยาเป็นระยะเวลานานขึ้นใน 30 วัน ผลการทดลองนี้ทำให้คาดว่า กลไกการออกฤทธิ์แก้ปวดของยาชนิดนี้ อาจมาจาก การปลดปล่อยเซโรโทนินที่เพิ่มสูงขึ้น ทำให้ ระดับเซโรโทนิน ที่ synaptic cleft เพิ่มสูงขึ้น ซึ่งอาจเป็นตัวชักนำให้เกิดการลดลงของตัวรับ 5-HT_{2A} receptor และ การเพิ่มขึ้นของตัวเก็บกลับเซโรโทนิน ในทางตรงกันข้าม การได้รับยาเป็นระยะเวลานานมากขึ้น อาจทำให้เซโรโทนิน ที่ถูกปล่อยออกมาหมดไป ระดับเซโรโทนิ นจึงลดต่ำลง และมีผลให้เกิดการปรับตัวที่ตัวรับ 5-HT_{2A} receptor และ ตัวเก็บกลับเซโรโทนิน ผลการทดลองนี้ เป็นหลักฐานที่สนับสนุนว่า กลไกการออกฤทธิ์แก้ปวดของพาราเซตามอล สามารถผ่านทางระบบประสาทส่วนกลางเซโรโทนิน

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สาขาวิชา สห.สง.ส.สรีรวิทยา.....
ปีการศึกษา 2540.....

ลายมือชื่อผู้สมัคร เนาวรัตน์ ชารททรัพย์.....
ลายมือชื่ออาจารย์ที่ปรึกษา
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

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Paracetamol is the most widely used analgesic drug . Although the mechanism of analgesic action of paracetamol is still not known, the involvement of central serotonin (5-hydroxytryptamine: 5-HT) system is one possibility. The antinociceptive effect of acute and chronic intraperitoneally (i.p.) administered paracetamol was assessed by tail flick latency measurements in the rat. A significantly increased tail flick latency was observed in acute and 15-day paracetamol-treated rats, but not in 30-day paracetamol-treated rats, at a dose of 400 mg/kg. By using platelets as a neuronal model, our results revealed a significant increase in platelet 5-HT content in 15-day paracetamol treated groups at a dose of 300 and 400 mg/kg compared to control groups (7024.67 ± 905.97 , 7342.83 ± 1041.35 and 3911.32 ± 438.07 ng/ 10^8 platelets, respectively, $p < 0.01$). A significant increase was also observed in platelet 5-HIAA content in 30-day paracetamol-treated groups at a dose of 300 and 400 mg/kg compared to control groups (13788.65 ± 2373.95 , 13866.77 ± 2517.06 and 7116.84 ± 1199.23 ng/ 10^8 platelets, respectively, $p < 0.01$). To investigate the plasticity of receptors at pre- and post synaptic membrane, we conducted a series of experiments by radioligand binding method on frontal cortex and brainstem membrane. The technique involved radioligand binding with [phenyl-4- 3 H]-spiperone and ketanserin for studying 5-HT $_{2A}$ serotonin receptor characteristics and [3 H]-imipramine and fluoxetine for studying 5-HT uptake sites characteristics. A significant decrease in the maximum number of 5-HT $_{2A}$ binding sites (Bmax) was demonstrated in all treatment groups with paracetamol 300 and 400 mg/kg on frontal cortex membrane, whereas the value of dissociation equilibrium constant (Kd) remained unchanged. An increase in the maximum number of 5-HT $_{2A}$ binding sites was observed in 30-day paracetamol-treated rats, compared with 15-day paracetamol-treated rats at a dose of 400 mg/kg (0.94 ± 0.01 and 1.34 ± 0.13 pmol/mg protein, respectively).

In contrast to 5-HT $_{2A}$ receptors, a significant increase in the maximum number of 5-HT uptake sites was demonstrated in all treatment groups with paracetamol 300 and 400 mg/kg on frontal cortex membrane, whereas the value of dissociation equilibrium constant remained unchange. A decrease in the maximum number of 5-HT uptake sites was observed in 30-day paracetamol-treated rats as compared with 15-day paracetamol-treated rats at a dose of 400 mg/kg (4.59 ± 0.52 and 2.71 ± 0.18 pmol/mg protein, respectively). Such changes of 5-HT $_{2A}$ receptors and 5-HT uptake sites was not observed on brainstem membranes. The post synaptic receptors became down-regulated whereas the uptake sites were up-regulated in frontal cortex membranes of acute and 15-day paracetamol treatments. These occurred concomitantly with an increase in platelet 5-HT level and antinociceptive activity. These abnormalities of receptors were normalizable after 30-day paracetamol treatments. From these results we suggest that down-regulation of 5-HT $_{2A}$ receptor in response to 5-HT release is a major step in mechanism underlying analgesia produced by this agent. Such increased 5-HT level at the synaptic cleft may induce up-regulation of 5-HT uptake sites. On the contrary, chronic use of paracetamol may result in 5-HT depletion which in turn produces re-adaptation of post-synaptic 5-HT $_{2A}$ receptor and pre-synaptic 5-HT uptake sites. These data provide further evidence for a central 5-HT dependent antinociceptive effect of paracetamol.

ภาควิชา..... สรีรวิทยา.....

สาขาวิชา..... สรีรวิทยา.....

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 จุฬาลงกรณ์มหาวิทยาลัย

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LIST OF ABBREVIATIONS

| | |
|-------------|--|
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| ATPase | adenosine triphosphatase |
| ASA | acetylsalicylic acid |
| B_{max} | maximum number of binding sites |
| $^{\circ}C$ | degree celsius |
| Ca^{2+} | calcium ion |
| $CaCl_2$ | calcium chloride |
| cAMP | cyclic adenosine monophosphate |
| cDNA | complementary deoxyribonucleic acid |
| Cl^{-} | chloride ion |
| CNS | central nervous system |
| 5-CT | 5-carboxamidotryptamine |
| DA | dopamine |
| DAG | diacylglycerols |
| DHBA | dihydroxybenzoic acid |
| DLF | dorsal lateral funiculus |
| DNA | deoxyribonucleic acid |
| DOB | 4-bromo-2,5-dimethoxyphenylisopropylamine |
| DOI | 1(2,5-dimethoxy-4-iodophenyl)-2-aminopropane |
| DR | dorsal raphe nucleus |
| ECD | electrochemical detector |
| EDTA | ethylenediaminetetraacetic acid |
| e.g. | exempli. gratia |
| g | gravity unit |
| G-protein | guanine nucleotide protein |
| GABA | gamma aminobutyric acid |
| GI | gastrointestinal |
| h | hour |
| $[^3H]$ | tritium |
| 5-HIAA | 5-hydroxyindolacetic acid |
| 5-HT | 5-hydroxytryptamine |
| 5-HTP | 5-hydroxytryptophan |
| HPLC | high performance liquid chromatography |
| IBS | imipramine binding sites |
| i.c.v. | intracerebroventricular |
| i.p. | intraperitoneal |
| IP_3 | inositol triphosphate |
| K^{+} | potassium ion |
| K_d | dissociation equilibrium constants |
| K_i | inhibitor constants |
| KCl | potassium chloride |
| μl | microliter |

| | |
|------------------------|--|
| LSD | lysergic acid diethylamide |
| μM | micromole |
| MAO | monoamine oxidase |
| Met | methionine |
| mg | milligram |
| MgCl_2 | magnesium chloride |
| min | minute |
| ml | milliliter |
| mM | millimolar |
| MR | median raphe nucleus |
| mRNA | messenger ribonucleic acid |
| N | normal concentration |
| nM | nanomolar |
| NaCl | sodium chloride |
| NA | noradrenaline |
| NMDA | N-methyl-D-aspartate |
| NRM | nucleus raphe magnus |
| NSAIDs | nonsteroidal anti-inflammatory drugs |
| NSE | neuron specific enolase |
| 8-OH-DPAT | 8-hydroxy-2-(di-n-propylamino)tetraline |
| PAG | periaqueductal gray matter |
| Para | paracetamol |
| PCPA | para-chlorophenylalanine |
| PGE_2 | prostaglandins type E_2 |
| $\text{PGF}_{2\alpha}$ | prostaglandins type $\text{F}_{2\alpha}$ |
| PGs | prostaglandins |
| PLA_2 | phospholipase A_2 |
| pmol | picomole |
| PRP | platelet rich plasma |
| RNA | ribonucleic acid |
| rpm | revolutions per minute |
| RVM | rostral ventromedial medulla |
| sec | second |
| SPA | stimulation produced analgesia |
| $T_{1/2}$ | half life time |
| Tr-OH | tryptophan hydroxylase |
| TrisHCl | Tris(hydroxymethyl)-aminomethane hydrochloride |
| Trp | tryptophan |