Cholinergically induced gamma oscillations are accompanied by rhythmic activity of the inhibitory network, which shunts excitatory synaptic inputs and sharpens the window in which pyramidal neurons can fire action potentials, leading to a tighter synchrony among pyramidal neurons (12). Thus, oseltamivir-induced population bursts may be shaped by the phasic activity of inhibitory interneurons, rather than pyramidal cells. Our preliminary data indicate that even at a high concentration of 1 mM, oseltamivir does not induce either depolarization or hyperpolarization of membrane potential of hippocampal excitatory neurons in primary dispersed cultures (n = 8, data not shown). Thus, our data imply two, but not mutually exclusive, possibilities concerning the action site of oseltamivir: i) inhibitory interneurons are an action target of oseltamivir, and ii) the effect of oseltamivir requires network activity flows, rather than single neurons. Given that two structurally unrelated neuraminidase inhibitors, that is, oseltamivir and NADNA, exerted the sane effect on network activity and that sialic acid, a neuraminidase substrate, regulates neurite adhesion between hippocampal neurons (1), we speculate that oseltamivir modulates sialylation-mediated neurite connectivity and enhances network synchronicity through interneurons.

Animal experiments with rodents demonstrate that orally (30 – 300 mg/kg) or intravenously (8 μmol/h per kg) administered oseltamivir accumulates in the brain via the blood-brain barrier, the brain-to-plasma concentration ratio ranging from 0.1 – 0.7 (roughly equal to 0.1 – 5 μM in the brain) (13, 14). Safety examinations of Tamiflu™ (oseltamivir), conducted by Roche, show that in 7 – 14-day-old rats, the brain concentration reaches more than 500 times greater than that in adult animals (see basic product information of Tamiflu™), suggesting a higher risk of a side-effect in younger brains. Interestingly, a minor allele with single nucleotide polymorphism in HsNEU2, which shows a strong binding affinity to oseltamivir, is frequently observed in Asians (9.29%), but not in Europeans and African Americans (15). This Asian population may be highly susceptible to oseltamivir and thus affected by neuropsychiatric disorders. Because our current data are not linked to behavioral alternations in human and animals, investigations in vivo will be necessary to examine whether oseltamivir-induced population bursts are related to some psychologic behaviors frequently seen in influenza-infected children.

Acknowledgments

This work was supported in part by a Grants-in-Aid for Science Research (No. 18021008, 17689004) from Japan Society for the Promotion of Science.

References

1 Cremer H, Chazal G, Carleton A, Goridis C, Vincent JD, Lledo PM. Long-term but not short-term plasticity at mossy fiber synapses is impaired in neural cell adhesion molecule-deficient mice. Proc Natl Acad Sci U S A. 1998;95:13242–13247.
2 Boyso A, Ayala J, Gutiérrez R, Hernández-RJ. Neuraminidase activity in different regions of the seizing epileptic and non-epileptic brain. Brain Res. 2003;964:211–217.
3 Isaev D, Isaeva E, Shatskih T, Zhao Q, Smits NC, Shworak NW, et al. Role of extracellular sialic acid in regulation of neuronal and network excitability in the rat hippocampus. J Neurosci. 2007;27:11587–11594.
4 Chavas LM, Tringali C, Fusi P, Venerando B, Tettamanti G, Kato R, et al. Crystal structure of the human cytosolic sialidase Neu2. Evidence for the dynamic nature of substrate recognition. J Biol Chem. 2005;280:469–475.
5 Izumi Y, Tokuda K, O'Dell KA, Zorumski CF, Narahashi T. Neuroexcitatory actions of tamiflu and its carboxylate. Asymmetric membrane ganglioside sialidase activity specifies axonal fate. Neurosci Lett. 2007;426:54–58.
6 Koyama R, Muramatsu R, Sasaki T, Kimura R, Ueyama C, Tamura M, et al. A low-cost method for brain slice cultures. J Pharmacol Sci. 2007;104:191–194.
7 Sasaki T, Matsuki N, Ikegaya Y. Metastability of active CA3 networks. J Neurosci. 2007;27:517–528.
8 Satoh N, Akiba T, Yokoshima S, Fukuyama T. A practical synthesis of (–)-oseltamivir. Angew Chem Int Ed. 2007;46:5734–5736.
9 Yamatsugu K, Kamijo S, Suto Y, Kanai M, Shibasaki M. A concise synthesis of tamiflu: third generation route via the diels-alder reaction and the curtius rearrangement. Tetrahedron Lett. 2007;48:1403–1406.
10 Ishii K, Hamamoto H, Sasaki T, Ikegaya Y, Yamatsugu K, Kanai M, et al. Pharmacologic action of oseltamivir on the nervous system. Drug Discov Ther. 2008;2:24–34.
11 Takahashi N, Sasaki T, Usami A, Matsuki N, Ikegaya Y. Watching neuronal circuit dynamics through functional multineuron calcium imaging (fMCI). Neurosci Res. 2007;58:219–225.
12 Fisahn A, Yamada M, Duttaroy A, Gan JW, Deng CX, McBain CJ, et al. Muscarine induction of hippocampal gamma oscillations requires coupling of the M1 receptor to two mixed cation currents. Neuron. 2002;33:615–624.
13 Morimoto K, Nakakariya M, Shirasaka Y, Kakinuma C, Fujita T, Tamai I, et al. Oseltamivir (Tamiflu™) efflux transport at the blood-brain barrier via P-glycoprotein. Drug Metab Dispos. 2008;36:6–9.
14 Ose A, Kusuhara H, Yamatsugu K, Kanai M, Shibasaki M, Fujita T, et al. P-glycoprotein restricts the penetration of oseltamivir across the blood-brain barrier. Drug Metab Dispos. 2008;36:427–434.
15 Li CY, Yu Q, Ye Z, Sun Y, He Q, Li XM, et al. A non-synonymous SNP in human cytosolic sialidase in a small Asian population results in reduced enzyme activity: potential link with severe adverse reactions to oseltamivir. Cell Res. 2007;17:357–362.

The 81st Annual Meeting of The Japanese Pharmacological Society
日本薬理学会年会，第81回（2008. 03. 17—19，横浜）

G0756052

P1L-40 Higenamine is the main active substance responsible for the inhibitory effect of *Nandina domestica* Thunberg on histamine-induced contraction of guinea pig tracheal smooth muscle

Takuro Ueki[1,2], Tatsuhiro Akaishi[1], Muneo Tsukiyama[1,3], Hiroko Kikuchi[3], Yoichi Yasuda[3], Tsuneo Morioka[2], Kazuho Abe[1]

[1]Lab. Pharmacol., Fac. Pharm., Musashino Univ., 1-1-20 Shinmachi, Nishitokyo-shi, Tokyo 202-8585, Japan, [2]TOKIWA Pharmaceut, Co. Ltd., Osaka 541-0052, Japan, [3]NOEVIR, Co. Ltd., Kobe 650-8521, Japan

We have previously reported that the crude extract from *Nandina domestica* Thunberg (NDE: 0.1-1 mg/ml) inhibits histamine-induced contraction of isolated guinea pig trachea and the inhibitory effect of NDE cannot be accounted for nantenine, a major alkaloid isolated from NDE. To identify the active constituent(s) responsible for the inhibitory effect of NDE on tracheal contraction, we obtained several fractions of NDE and investigated their pharmacological effects on contractile responses in isolated guinea pig trachea. Among five fractions prepared from NDE by HP-20 column chromatography, only the 40% methanol fraction inhibited histamine-induced tracheal contraction. The 40% methanol fraction was further analyzed with the ultraviolet spectrometer and liquid chromatograph/mass spectrometer. Finally, we obtained only one fraction that inhibited histamine-induced tracheal contraction, and the mass spectrometry and nuclear magnetic resonance analysis identified higenamine as the active substance. We conclude that higenamine is the main active constituent of NDE in inhibiting tracheal contraction.

P1L-41 Effects of pressure stimulus on cell proliferation and differentiation in L6 skeletal muscle cells

Yoshiki Fujisawa[1], Kenji Iizuka[1], Noriteru Morita[2], Takuji Machida[1], Masahiko Hirafuji[1]

[1]Dept. Pharmacol., Facul. Pharm. Sci., Health Sci. Uni. of Hokkaido, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido 061-0293, Japan, [2]Dept. Wellness Planning., School of Lifelong Learning Support Systems, Hokusho Uni., 23 Bunkyodai, Ebetsu, Hokkaido 069-8511, Japan

Mechanical forces related to pressure is an important factor for cell hypertrophy and proliferation. The effects of pressure stimuli on skeletal muscles are not yet well characterized. The purpose of this study is to examine the effects of a pure pressure stimulus on skeletal muscle cells. Atmospheric pressure was applied to rat L6 myoblasts and myotubes at 160 mmHg for 3 hours. Protein and mRNA expressions were analyzed by using immunoblotting and real-time RT-PCR, respectively. Phosphorylated ERK and JNK were both increased in pressurized skeletal muscle myoblasts. Phosphorylated p38, myogenin protein and insulin-like growth factor mRNA were all decreased in pressurized skeletal muscle myoblasts. Induction of cell differentiation to myotubes resulted in an increase of phosphorylated ERK compared to myoblasts. However, pressurization to myotubes failed to induce significant change in phosphorylated ERK. These findings demonstrate that a pure mechanical pressure stimulus enhances cell proliferation and suppresses cell differentiation in skeletal muscle myoblast, and raise the possibility that elevated intramuscular pressure may have diverse effects according to the differentiative stage of skeletal muscle.

D16575

P2I-01 Oseltamivir induces spike synchronization in hippocampal networks

Atsushi Usami[1], Takuya Sasaki[1], Nobuhiro Satoh[2], Takahiro Akiba[3], Satoshi Yokoshima[2], Tohru Fukuyama[2], Kenzo Yamatsugu[3], Motomu Kanai[3], Masakatsu Shibasaki[3], Norio Matsuki[1], Yuji Ikegaya[1]

[1]Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Tokyo University, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, [2]Laboratory of Synthetic Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Tokyo University, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, [3]Laboratory of Synthetic Organic Chemistry, Graduate School of Pharmaceutical Sciences, Tokyo University, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Oseltamivir is an antiviral drug used to treat influenza. It inhibits neuraminidase, thereby preventing influenza virus from emerging from infected cells. The effect of oseltamivir on the central nervous system is largely unknown, however. We monitored the activity of neurons treated with oseltamivir in hippocampal slice cultures, by using electrophysiological recordings and functional multineuron calcium imaging. Double patch-clamp recordings revealed that oseltamivir led to spike synchronization among adjacent hippocampal CA3 neurons. To investigate how oseltamivir alters neuronal network operation, we simultaneously recorded the spike activity of hundreds of hippocampal neurons. Oseltamivir and its active form both induced global spike synchronization that recruited virtually all neurons in the network and persisted for more than several seconds. The effect was concentration-dependent. Network excitability may be regulated by extracellular sialic residues through neuraminidase.

P2I-02 Visualization of neuronal network activity: implications for a new drug-screening method in systems neuropharmacology

Naoya Takahashi, Takuya Sasaki, Norio Matsuki, Yuji Ikegaya

Lab. Chem. Pharmacol., Grad. Sch. Pharm. Sci. Univ. Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Synchronization in cortical networks is a prevalent feature that reflects dynamic processing of sensory input and internal information. We developed high-speed functional multineuron calcium imaging (fMCI) to simultaneously record action potentials from about hundreds of neurons at up to 2000 frames/s and reconstructed the spatiotemporal pattern of hippocampal CA3 network activity in vitro. Spontaneous activity displayed the emergence and dynamics of synchronized network activity with millisecond coordination. Whole-cell recordings from synchronized neuron pairs revealed that coordinated excitatory inputs potentially contributed to precise synchronization. To elucidate the profile of network synchrony, in fMCI data sets, we estimated the statistical significance of synchronicity between all possible neuron pairs. The graphs, in which individual neurons were functionally connected based on strength of pairwise correlation, were sparse, complex and locally clustered, exhibiting small-world architectures. These findings provide a simplified strategy for evaluating network operations, which will provide a new drug screening technique in systems neuropharmacology.

28J-am06

ラット脳および培養アストロサイトにおけるTissue Inhibitor of Matrixmetalloproteinase発現に対するエンドセリンの作用

○小山 豊[1]、田中 一裕[1]（[1]大阪大谷大薬）

【目的】Matrix Metalloproteinases(MMPs)は、細胞外マトリクス分子の分解を行う分泌型プロテアーゼファミリーで、脳病態時の脳浮腫や神経細胞死の発生に関わる。一方、内因性の MMP 阻害因子として見いだされた Tissue Inhibitor of Matrixmetalloproteinase (TIMP)も脳病態時に発現が増加する。この増加した TIMP は、過剰な MMP 活性を抑制し、傷害から脳を保護すると考えられている。エンドセリン（ET）は、神経系の病態生理反応に関わる因子である。我々は既に、ET_B 受容体アゴニストが、ラット脳内の MMP2 および MMP9 の発現を増加させることを報告している。今回、ラット脳での TIMP 発現に対する ET の作用を検討した。【方法】ラット脳室内への ET アゴニスト持続投与はミニ浸透圧ポンプを用い行なった。TIMP の mRNA およびタンパク量の測定は定量的 RT-PCR 法およびイムノブロットにより測定した。培養アストロサイトは生後 0−2 日齢の Wistar 系ラット大脳皮質より調製した。【結果】ET_B アゴニスト Ala-ET-1(500pmole/day)を 7 日間脳室内へ持続投与したラットの大脳では TIMP-1 および TIMP-3 mRNA の増加が、海馬および線条体では TIMP-1 mRNA の増加が観察された。免疫組織化学的検討は、これらの TIMP が GFAP 陽性アストロサイトで発現していることを示した。培養アストロサイトに対し ET- 1 (100nM)は、TIMP-1 および TIMP-3 mRNA 発現、および細胞外へ遊離を促進した。【考察】以上の結果は、脳病態時のアストロサイトの TIMP 産生における、ET の関与を示唆する。

28J-am07

ブラジキニン(BK)による逆モードNa^+-Ca^{2+}交換機構(NCX)の活性化及びB_1受容体を介するミクログリアの遊走性・化学走性増加

○井福 正隆[1]、Katrin Farber[2]、奥野 祐子[1]、山川 裕希子[1]、宮本 奈貴[1]、Christiane Nolte[2]、和田 圭司[1]、Helmut Kettenmann[2]、野田 百美[1]（[1]九大院薬、[2]MDC、[3]国立精神・神経セ）

【目的】中枢神経系で免疫系を司るミクログリアは、様々な神経変性疾患や傷害、虚血時に活性化され、また炎症とも深く関わっていることが知られている。我々は、炎症性メディエーターであるブラジキニン(BK)の受容体がミクログリアにも発現しており、Ca^{2+}依存性の K^+電流 ($I_{K(Ca)}$) を誘発することを報告してきた。$I_{K(Ca)}$ の誘発はミクログリアの遊走性増加に関与するという報告があるので、BK によるミクログリアの遊走性・化学走性への関与及びその要因について検討した。【方法】初代培養ミクログリアは生後 3 日目の Wister ラットまたは BK 受容体ノックアウト(KO) マウス、Na^{2+}/Ca^{2+}交換機構タイプ 1 （NCX1）KO マウス（ヘテロ）の大脳皮質より単離した。遊走性は、長期培養観察装置を、化学走性は Boyden chamber を用いて解析した。【結果・考察】BK 処置により、遊走性・化学走性の増加が見られ、これらの反応は B_1 受容体の拮抗薬により抑制され、B_1-KO マウスから得られたミクログリアでは消失した。BK による遊走性・化学走性増加はプロテインキナーゼ C (PKC)・$I_{K(Ca)}$ の阻害薬、逆モード NCX1（Ca^{2+}流入モード）の特異的な拮抗薬および NCX1-KO マウスより単離したミクログリアで有意に抑制された。また *in vivo* においても、傷害部位へのミクログリアの集積は、$I_{K(Ca)}$の阻害薬及び B_1-KO マウスで有意に減少していた。以上の結果より、BK によるミクログリアの遊走性・化学走性増加および傷害部位への集積のメカニズムは B_1 受容体を介した PKC の活性化と逆モード NCX1 の活性化による細胞内への Ca^{2+}の流入、及びそれによる $I_{K(Ca)}$の誘発を介することが明らかとなった。このようなシグナリングを介したミクログリアの集積が傷害部位でどのような役割を演じているのかを検討するのが今後の課題である。

G0756444

28J-am08

ラットの睡眠覚醒サイクルに対する kavain の影響

○井 隆興[1]、四宮 一昭[1]、武田 康宏[1]、亀井 千晃[1]（[1]岡山大院医歯薬）

【目的】現在臨床で繁用されている benzodiazepine 系睡眠薬は、様々な副作用を有し、睡眠の質を低下させることが報告されている。従って、新規睡眠薬として、生薬抽出成分が注目されている。Kava-kava はその抽出成分が睡眠作用を有することが判明している生薬である。しかし長期服用により劇症肝炎を発症されることが知られており、国内においてその使用が制限されている。そこで今回、kava-kava 中に含まれる成分のうち；肝炎発症に関与していないとされている kavain に注目し、睡眠覚醒サイクルに対する効果を rilmazafone および diphenhydramine の効果と比較検討した。【方法】右前頭葉皮質および頸部筋に慢性電極を挿入したラットを用い、薬物投与後の脳波および筋電図を 6 時間測定した。睡眠ステージの分類および脳波の周波数解析には、睡眠解析プログラムである SleepSign ver.2.0 を用いた。【結果】Kavain, rilmazafone および diphenhydramine は睡眠導入潜時を有意に短縮させた。また kavain および rilmazafone では有意な覚醒時間の短縮作用および NREM 睡眠時間の延長作用が確認されたが、diphenhydramine では認められなかった。次に NREM 睡眠時間を延長した kavain および rilmazafone の睡眠の質 (delta activity) に対する影響を検討した。その結果、kavain は delta activity を有意に上昇させたが、rilmazafone は逆に有意に低下させた。【考察】Diphenhydramine は睡眠導入作用のみ有することが明らかとなった。一方 kavain は、rilmazafone と同様の睡眠パターンを有するが、rilmazafone とは異なり、睡眠の質をも改善する化合物である可能性が示唆された。

日本薬学会，第１２８年会
（２００８．０３．２６−２８，横浜）

28J-am09

強制水泳によって誘導されるうつ様症状に対するLeu-Ileの効果

○日比(古川) 陽子[1,2]、新田 淳美[2]、池田 武史[3]、難下 幸治[3]、山田 清文[2]、鍋島 俊隆[4]（[1](財)長寿科学振興財団、[2]名大病院薬、[3]協和発酵、[4]名城大薬）

（目的）近年、多くの人々がうつ病に罹患しており、社会問題にもなりつつある。うつ病患者の脳では海馬の縮小がみとめられるなど、神経変性や神経新生の異常が起こっていることが示唆されている。一方、我々はこれまでに球状性ジペプチド Leu-Ile が様々な神経変性精神疾患に効果を示すことを示してきた。そこで今回、Leu-Ile が抗うつ作用を示すのではないかと考え、マウスにうつ様症状を誘導するモデルを用いて Leu-Ile の効果を調べた。

（方法）雄性 ICR マウスを水を張った円筒形の容器に入れ、毎日 6 分間の強制水泳試験を 2 週間連続して行った。投入直後のマウスは水槽から逃れようと泳ぐが次第に浮いているだけの無動状態となる。この無動時間の長短はうつ様症状の指標とされており、抗うつ薬投与によって短縮する。そこで Leu-Ile を強制水泳直後に連続経口投与し、無動時間に及ぼす影響を調べた。

（結果および考察） 2 週間の強制水泳によってマウスの無動時間は著しく増大し、うつ様症状が誘導されたことが確認された。Leu-Ile を 750 μmol/kg 投与したマウス群では、投与開始 10 日前後から無動時間がコントロール群に比べて有意に短縮した。自発行動量は Leu-Ile 投与マウスとコントロールの間に差はなかった。Real time RT-PCR の結果から、Leu-Ile 連続投与によって海馬と前頭皮質において BDNF の発現量が増大することが示された。さらに、BrdU の取り込みを指標として細胞新生について検討したところ、2 週間の強制水泳によって海馬歯状回における BrdU 陽性細胞数が著しく減少したが、Leu-Ile 投与によって、この細胞数減少は抑制された。以上の結果より、Leu-Ile が BDNF シグナル伝達経路を介してストレスによる細胞増殖抑制を改善し、抗うつ作用を示す可能性が示唆された。

D16576

28J-am10

リン酸オセルタミビア(タミフル)とその生体内活性体のラット脳モノアミン神経伝達系におよぼす影響

○佐藤 かな子[1]、野中 良一[1]、小豚 昭夫[1]、中江 大[1]、上原 眞一[1]（[1]東京都健安研）

【目的】インフルエンザ治療薬である、リン酸オセルタミビア（タミフル）服用者に、近年、飛び出し、転落等の異常行動が報告されたため、現在、厚生労働省より、「10 代の患者にはタミフルの処方を原則中止」の方針が示されている。しかし、タミフル服用と異常行動との因果関係は、いまだ明らかになっていない。タミフルはラットの血液脳関門を通過することが報告[1]されており、このことはタミフル服用時の異常行動が覚醒剤や麻薬と類似した機序で発生することを示唆する。タミフルは、プロドラッグであり、生体内で代謝され活性体（GS4071）となり作用を発揮する。そこで、本報告は、タミフル服用と異常行動との関連性の有無を明らかにすることを目的に、タミフルとGS4071 の脳モノアミン神経伝達系の前シナプス側における 3 種類の神経伝達物質（ドーパミン、セロトニン、ノルエピネフリン系）および後シナプス側におよぼす影響を試験管内試験で検索した。【方法】ラット脳より前シナプス側および後シナプス側のシナプトソームをそれぞれ調整した。これらにタミフルまたは GS4071 を作用させた時、前シナプス側における神経伝達物質の再取り込み阻害および遊離促進作用と後シナプス側における G タンパク活性化への影響を覚醒剤および麻薬等と比較検討した。【結果および考察】タミフルおよびGS4071 は、前シナプスにおける 3 種類の神経伝達物質の再取り込み阻害と遊離促進作用、および後シナプスでのGタンパク活性化のいずれにも影響しなかった。[2]したがって、タミフル服用時における異常行動の発生機序には、脳モノアミン神経伝達系の変化が関与しないものと示唆された。

[1] http://www.fda.gov/medwatch/SAFETY/2003/tamiflu_deardoc.pdf

[2] Satoh, K., *et al.*, *Biol. Pharm. Bull.*, 30 (9), 1816 (2007)

G0756447

28J-am11

モルヒネ誘発嘔気・嘔吐および精神依存形成に対する非定型抗精神病薬アリピプラゾール（エビリファイ®）の効果

○塩川 満[1,2]、成田 年[1]、武井 大輔[1]、鵜川 百合[1]、中郁 篤史[1]、橋本 敬輔[1]、忍巻 恵子[1]、鈴木 雅美[1]、井上 忠夫[1]、鈴木 勉[1]（[1]星薬大・薬・薬品毒性、[2]聖路加国際病院薬）

モルヒネはがん疼痛緩和で有用な薬剤であるが、副作用として嘔気・嘔吐を発現させるためその予防に中枢性ドパミン受容体拮抗薬が中心に処方される。しかし中枢性ドパミン受容体拮抗薬には副作用として錐体外路症状が発現するため、より副作用の少ない非定型抗精神病薬が適している。非定型抗精神病薬アリピプラゾールは既存の抗精神病薬とは異なり、ドパミン D2 受容体部分作動性を有することからドパミン作動性神経伝達が過剰な場合には、ドパミン D2 受容体の拮抗薬として作用し、ドパミン作動性神経伝達が低下している場合には、ドパミン D2 受容体の作動薬として作用することが基礎実験で確認されている。そこで本研究ではモルヒネ誘発ドパミン関連行動に対する非定型抗精神病薬アリピプラゾールの効果を検討した。

フェレットを用いたモルヒネ誘発嘔気・嘔吐は、アリピプラゾールの前処置により、有意に抑制された。また、モルヒネ誘発ドパミン関連行動である報酬効果ならびに自発運動促進作用はアリピプラゾール処置により用量依存的かつ有意に抑制された。また、モルヒネ誘発嘔気・嘔吐を抑える用量のアリピプラゾールの処置では、錐体外路症状の指標となるカタレプシーは観察されなかった。さらに、モルヒネにより誘発される G 蛋白質活性化作用はアリピプラゾール処置により変化が認められなかったが、ドパミン誘発 G 蛋白質活性化作用は有意に抑制された。これらのことから、アリピプラゾールは μ受容体刺激には直接影響を与えず、ドパミン受容体を拮抗することにより、嘔気・嘔吐や精神依存形成などのドパミン関連行動のみを抑制し、さらには錐体外路症状がほとんど現れない非常に有用なオピオイドとの併用薬物である可能性が示唆された。

Effects of Oseltamivir Phosphate (Tamiflu) and Its Metabolite (GS4071) on Monoamine Neurotransmission in the Rat Brain

Kanako SATOH,* Ryouichi NONAKA, Akio OGATA, Dai NAKAE, and Shin-ichi UEHARA

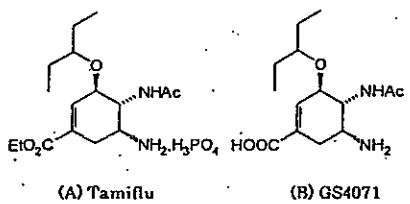
Department of Environmental Health and Toxicology, Tokyo Metropolitan Institute of Public Health; 3–24–1 Hyakunincho, Shinjuku-ku, Tokyo 169–0073, Japan.

Received June 19, 2007; accepted July 10, 2007; published online July 10, 2007

As abnormal behaviors such as jumping and falling from balcony were reported in patients aged 10 to 19 years who administrated oseltamivir phosphate (Tamiflu) for treatment influenza infection, the Ministry of Health, Labor and Welfare in Japan notified that, as a rule, Tamiflu should not be prescribed to patients aged 10 to 19 years. To examine the relationship between Tamiflu and abnormal behaviors, we investigated the effects of Tamiflu and its carboxylic acid metabolite, GS4071, on the central nervous system, that is, on 3 neurotransmitters (dopamine, serotonin, and norepinephrine) in presynapses (inhibition of re-uptake, promotion of release) and postsynapses (guanosine 5′-triphosphate (GTP) γS binding), using rat brain synaptosomes. Neither Tamiflu nor GS4071 influenced the re-uptake/release of the 3 monoamines or GTP binding in postsynapses.

Key words Tamiflu; oseltamivir phosphate; GS4071; dopamine; serotonin; norepinephrine

As an anti-influenza virus agent, oseltamivir phosphate (ethyl-(3*R*,4*R*,5*S*)-3-(1-ethylpropyloxy)-4-acetamido-5-amino-1-cyclohexane-1-carboxylate phosphate, Fig. 1A) (proprietary name: Tamiflu®) was developed by Roche Laboratory Inc. (Switzerland). In Japan, the Ministry of Health, Labor and Welfare approved this agent in 2000. In February 2001 and July 2002, Chugai Pharmaceutical Co., Ltd., as a Japanese agency, started the sales of 75-mg Tamiflu® capsules and 3% Tamiflu® dry syrup, respectively. The action mechanism of Tamiflu is reported as follows[1]: it suppresses viral release from the surfaces of infected cells by inhibiting neuraminidase, an enzyme essential for the proliferation of type A/B influenza virus, preventing viral infection/proliferation in other cells. This mechanism is similar to that of zanamivir hydrate (Relenza®, anti-influenza virus agent). Relenza is an inhalation agent, whereas Tamiflu is an oral preparation; therefore, the administration method is simpler, although the interval until Tamiflu reaches the infected site is prolonged. Furthermore, amantadine hydrochloride (Symmetrel®) is also administered as an oral anti-influenza virus agent. However, it is not effective for influenza infection other than type A influenza infection. Thus, Tamiflu can be simply administered to treat influenza infection, and may be useful for preventing and treating bird influenza infection. According to Roche Laboratory Inc., the sales situation of Tamiflu is as follows.[2] Japanese patients (n=34000000) account for approximately 75% of the total world Tamiflu consumption (45000000 persons, as of March 12, 2007). American patients comprise the second highest percentage (20%). The amount of Tamiflu administered to children in Japan was 13 times higher than that in the United States. The usage of Tamiflu in Japan is numerous.



(A) Tamiflu (B) GS4071

Fig. 1. Chemical Structures
A: Tamiflu (oseltamivir phosphate), B: GS4071.

In 2007, abnormal behaviors such as jumping and falling were reported in 10- to 19-year-old patients administrated Tamiflu. Therefore, the Ministry of Health, Labor and Welfare in Japan notified that, as a rule, Tamiflu should not be prescribed to patients aged 10 to 19 years. On April 25, 2007, the Ministry of Health, Labor and Welfare published the "Reports on the Side Effects of Oseltamivir Phosphate (Tamiflu)",[3] which had been submitted between the start of sales and March 20, 2007. According to the report, abnormal behaviors were observed in 186 of 1268 patients with side effects (8 of them died). In the presence of influenza encephalopathy, abnormal behaviors similar to those after Tamiflu administration have also been reported. In Japan (1999, 2000), encephalopathy frequently develops in children aged less than 6 years (2.5/100000 persons),[4,5] and the mortality rate (10 to 30% of patients with encephalopathy) and incidence of squeal (approximately 20% of them) are high.[4,6] In children aged over 1 year, the side effects of Tamiflu are rare and slight.[7] A study indicated that there was no association between Tamiflu and mortality/encephalopathy in infants aged less than 1 year.[8] Concerning the relationship between Tamiflu and abnormal behaviors, another study reported that there was no significant difference in the incidence of abnormal behaviors between patients with and without Tamiflu (11.9% *vs.* 10.6%, respectively).[9]

To evaluate psychoactive drug activity quickly, we reported an assay system for investigating the influence on the central nervous system using synaptosomes prepared from the rat brain[10]: a system for examining the influence of various chemicals including psychoactive drugs on 3 neurotransmitters (dopamine (DA) system, serotonin (5HT) system, and norepinephrine (NE) system) in presynapses (inhibition of re-uptake; promotion of release). Many of the monoamine receptors, including DA, 5HT, and NE receptors, are considered to belong to the superfamily of guanosine 5′-triphosphate (GTP) binding protein-coupled receptors in postsynapses (GTP binding). Abnormal behaviors seen in the case of Tamiflu administration to treat influenza closely resemble those seen in the case of the acute psychoactive drugs intoxication, a pleasurable mix of stimulant-like and hallucinogen-like effects. Oral administration of Tamiflu, an ethyl ester prodrug, is converted to the active form, (3*R*,4*R*,5*S*)-3-(1-ethylpropyloxy)-4-acetamido-5-amino-1-cyclohexane-1-carboxylic acid (GS4071, Fig. 1B) *in vivo*.[1] As an animal experiment it is reported that Tamiflu passes the brain barrier,[11] we investigated the influence of Tamiflu and its metabolite GS4071, which were supplied by Prof. Shibasaki (the Uni-

* To whom correspondence should be addressed. e-mail: Kanako_Satou@member.metro.tokyo.jp

versity of Tokyo, Tokyo, Japan), on DA, 5HT, and NE as well as GTP binding using a psychoactive drug-assay system to examine the relationship between Tamiflu and abnormal behaviors.

MATERIALS AND METHODS

Reagents Tamiflu and GS4071 were a kind gift from Prof. Shibasaki. They were synthesized according to Fukuta *et al.*[12] and Mita *et al.*[13] Methamphetamine (MAP) and cocaine were purchased from Takeda Pharmaceutical Company Limited and Dainippon Sumitomo Pharma Co., Ltd., respectively. Chemicals were dissolved in dimethyl sulfoxide (DMSO, final concentration: 0.1%). ^{3}H-DA (2.20 TBq/mmol), ^{3}H-5HT (1.11 TBq/mmol), ^{3}H-NE (1.93 TBq/mmol), and [^{35}S]GTPγS (46.25 TBq/mmol) were purchased from PerkinElmer Inc. (MO, U.S.A.). Other reagents used in the study were of the highest grade commercially available.

Animals Male Sprague Dawley rats (crlj:CD (SD)) at 5 weeks old were obtained from Charles River Japan (Kanagawa, Japan). After the rats were preliminary bred for one week, they were killed under ether anesthesia and their brains were quickly removed.[10,14] All animal studies were performed in accordance with the UFAW Handbook on the Care and Management of Laboratory Animals.

Preparation of Cerebral Synaptosomes for Re-uptake and Release Assay The striatum and cerebral cortex were dissected from the rat brain. Crude synaptosomes were obtained by the methods described in our previous reports.[10,14] The crude synaptosome from the striatum was used for the assay of re-uptake and release of DA, and that from the cortex was used for the assays of 5HT and NE. For the release assay, 1 μM reserpine was added to 0.32 M sucrose and buffer. The re-uptake and release assays were started immediately after the preparation of synaptosomes. Protein concentrations were determined by the modified Lowry method using a Bio-Rad assay kit.

Preparation of Cerebral Synaptosomes for GTPγS Binding Assay The whole brain dissected on ice was homogenized. Crude synaptosomes were obtained by the modified methods described in previous[15] and our personal reports. The synaptosome was divided into aliquots and stored at −80 °C until use.

^{3}H-DA, ^{3}H-5HT, and ^{3}H-NE Re-uptake and Release Assays The re-uptake and release assays were conducted using the methods described in our previous reports.[10,14] The final concentration of 0.1% DMSO had no effect on the activity. Specific uptake or release was calculated by subtracting the non-specific uptake (DA; 260, 5HT; 500, NE; 720 dpm) or release (DA; 5210, 5HT; 4960, NE; 2200 dpm) content from the total uptake (DA; 11600, 5HT; 2960, NE; 8480 dpm) or release (DA; 10160, 5HT; 7560, NE; 6770 dpm) content. From these results, the drug concentration giving the IC_{50} or EC_{50} was obtained.

[^{35}S]GTPγS Binding Assay The GTP binding assay was determined by the methods modified in previous[16] and our personal reports. Specific monoamine- or chemical-stimulated [^{35}S]GTPγS binding values were calculated by subtracting basal binding values (obtained in monoamine or chemical absence; 900 cpm) from stimulated values (obtained in monoamine or chemical presence). The % of 5-HT maxima was determined by dividing DA-, NE-, or chemical-induced maximal binding using the 5-HT-stimulated maximal binding value (2110 cpm) as a reference compound.

Statistical Analysis IC_{50} and EC_{50} values were determined using the sigmoidal dose-response curve fitting obtained by a software, KaleidaGraph ver. 4 (Synergy Software, PA, U.S.A.). The data represented the mean values of three independent experiments (n=3).

RESULTS AND DISCUSSION

In this study, we examined that Tamiflu and GS4071 on DA, 5HT, NE-reuptake and release assays, and GTP binding assay using rat brain synaptosomes. We compared the influence of Tamiflu and GS4071 on ^{3}H-DA, ^{3}H-5HT, and ^{3}H-NE re-uptake with that of a stimulant, MAP, and a narcotic, cocaine (Figs. 2A—C). Both MAP and cocaine potently inhibited DA, 5HT, and NE re-uptake, and their IC_{50} values were similar to those previously reported.[10] However, neither Tamiflu nor GS4071 influenced re-uptake of the 3 monoamines. In release assay, MAP potently promoted DA/NE release, but cocaine did not influence the 3 monoamines. MAP's EC_{50} values and the finding of cocaine were consistent with the results of our previous study.[10] Neither Tamiflu nor GS4071 promoted the release of ^{3}H-DA, ^{3}H-5HT, and ^{3}H-NE (Figs. 2D—F). Subsequently, we studied a [^{35}S]GTP binding assay under conditions which facilitated the adequate responses of DA, 5HT, and NE. Neither MAP nor cocaine promoted G-protein binding, as previously reported. Also, neither Tamiflu nor GS4071 bound the G-protein binding of DA, 5HT, and NE receptors (Fig. 3).

In a symposium held by the Japanese Society of Pharmacological Epidemiology (JSPE) (May 20, 2007), some investigators reported that there was no association between Tamiflu administration and abnormal behaviors based on statisti-

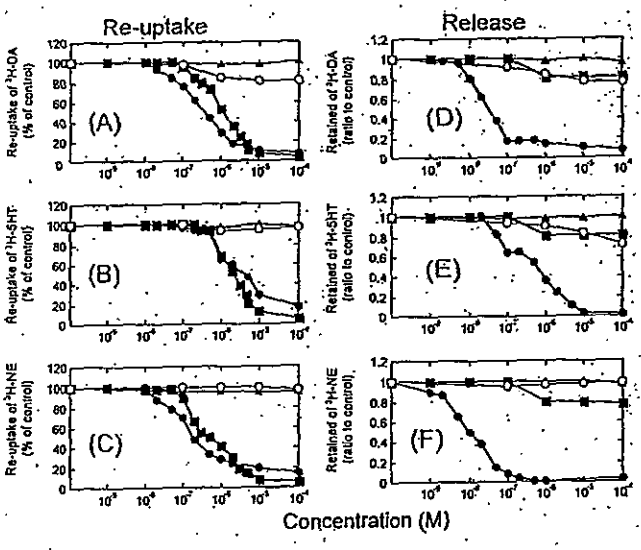


Fig. 2. Inhibition of Re-uptake and Stimulation of Release of Monoamines by Tamiflu and GS4071

The synaptsome fraction prepared from striatum was used for the assay of dopamine (A and D), and that prepared from the cortex was used for the assay of 5-HT (B and E) and norepinephrine (C and F). The S.D. values are less than 4.0%. (A), (B), and (C): re-uptake assay; (D), (E), and (F): release assay. ▲: Tamiflu; ○: GS4071; ●: methamphetamine; ■: cocaine.