行政院國家科學委員會專題研究計畫　成果報告

慢性產前嗎啡注射對新生幼鼠腦部血清素分泌的影響及血清素再吸收抑制劑對新生幼鼠嗎啡斷癮症狀的治療效果

計畫類別：個別型計畫
計畫編號：NSC92-2314-B-038-023-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：臺北醫學大學醫學研究所

計畫主持人：葉健全

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 93 年 11 月 2 日

行政院國家科學委員會補助專題研究計畫 ˇ 成 果 報 告
□期中進度報告

(計畫名稱)

慢性產前嗎啡注射對新生幼鼠腦部血清素分泌的影響及血清素再吸收抑制劑對新生幼鼠嗎啡斷癮症狀的治療效果

計畫類別：x 個別型計畫　　□ 整合型計畫

計畫編號：NSC 92－2314－B－038 －023

執行期間：　92 年　8 月 1 日至　93　年　7　月 31　日
計畫主持人：葉健全
共同主持人：
計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：□精簡報告　x 完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份
處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：台北醫學大學醫學研究所

中　華　民　國　93　年　10　月　30　日

中文摘要

我們對懷孕雌性大白鼠於受孕前至生產後此期間予以每日皮下施打嗎啡，而後偵測三種血清素再吸收抑制劑是否會抑制所生五天大幼鼠因施打 naloxone 引發之嗎啡戒斷症狀。此三種血清素再吸收抑制劑分別為 citalopram、chlomipramine 及 fluoxetine。所觀測之幼鼠之 naloxone 引發之嗎啡戒斷症狀有兩種即 yawning 及 abdominal stretching。皮下單一施打此三種藥物均有效能抑制 yawning 及 abdominal stretching。 但是對抑制戒斷症狀之效價之排列為 citalopram , chlomipramine >> fluoxetine。 此效價之比量皆僅此三種藥物對血清素再吸收轉子之親合力， 因此本實驗提供使用血清素再吸收抑制劑來治療嬰幼兒嗎啡戒斷症狀。

Abstract

Previous investigations had shown that inhibitor of serotoinin reuptake transporter could attenuate morphine withdrawal syndrome in adult animals. In the present study, we determined whether that enhance serotonin-mediated neurotransmission by serotonin reuptake inhibitors is able to attenuate the expression of the naloxone-precipitated morphine withdrawal syndrome in neonatal Sprauge-Dawley rat born to dams rat received morphine injection since a week before mating till 5 days after delivery. Withdrawal syndrome of morphine, manifested as frequent abdominal stretching and yawning, was generated by injection of naloxone on postnatal day 5. Pre-injection with SRET inhibitors, fluoxetine, clomipramine or citolopram, significantly attenuated the naloxone-precipitated syndrome in a dose-dependent manner without apparent side effect. The rank order of inhibitory potency is citalopram= clomipramine > fluoxetine. This result suggests that inhibitor of SERT may be of potential in treating neonatal morphine withdrawal syndrome.

Key words: morphine withdrawal syndrome, neonatal rat, SERT inhibitor, naloxone

Serotonin reuptake inhibitors attenuating morphine withdrawal syndrome in neonatal rate passively exposed to morphine

Chi-Chun Wu[a,b], Julia Yi-Ru Chen[c,d], I-An Chen[e], Geng-Chang Yeh[c,d,e*]

[a]Department of Anesthesiology, Taipei Medical University Hospital,
[b]Department of Anesthesiology, School of Medicine, Taipei Medical University
[c]Department of Pediatrics, Taipei Medical University Hospital
[d]Department of Pediatrics, School of Medicine, Taipei Medical University
[e]Graduate Institute of Medical Science, Taipei Medical University

Corresponding to Geng-Chang Yeh
Department of Pediatrics, Taipei Medical University Hospital
No 252 Wu-Sin ST. Taipei Taiwan 110
Tel:011-886-2-27372181 ext 3320
Fax:011-886-2027360399
e mail : cmbyeh@tmu.edu.tw

1. Introduction

Infant born to mothers addicted to morphine or heroin during pregnancy have great chance to produce acute withdrawal syndrome after birth. The withdrawal symptoms include jitteriness, irritability, shrill crying, hyperactivity-hypertonicity, poor feeding, vomiting, diarrhea, sneezing, tachypnea, and seizure (Volpe, 1995). Most of the newborns presenting apparent features of withdrawal syndrome require intensive care. In addition to supportive therapy to stabilize the vital sign, drug therapy with paregoric, phenobarbital, chlorpromazine or diazepam is frequently used to minimize the symptoms of central nervous and gastrointestinal systems. However, the side effects of these applied medication in CNS required careful monitoring. Thus, to improve the treatment outcome for neonatal morphine withdrawal syndrome development of new effective and safety medication is still needed

Serotonin (5-hydroxytyramine, 5-HT), an important biogenic amine, exerts its neurotransmission via activating different 5-HT subtype receptors in the CNS. Serotonin is synthesized from tryptophan through the action of tryptophan hydroxylase, and is released by prenaptic stimulation. Its action is terminated by uptaking through presynaptic serotonin reuptake transporter (SERT), which is a transmembrane protein uptaking serotonin by a sodium-dependent mechanism. Serotonin once been uptaked will be reused or degraded into hyrodroxyindoleactic acid (5-HIAA). Serotonin-mediated neurotransmission has long been implicated in the regulation of wide variety of cortical functions including modulation of appetite, memory, mood, emotionality, thermoregulation, and sexual behavior (Jacobs and Azmitia, 1992). The forebrain and spinal serotonin pathway are known to be involved in pain inhibition and morphine analgesia (Brase, 1979). It has been shown that deficiency in serotonin-mediated neurotransmission contributes to the expression of major depression and chronic pain (Archer et al., 1986), and therefore, selective SERT inhibitor such as citalopram, fluoxetine, and paroxetine are widely used in the treatment of depression, anxiety disorder, eating disorder, obsessive-compulsive disorder and substance abuse (Murphy, 1990; Singh et al., 2001). Previous reports had shown that acute administration of morphine could enhance brain serotonin synthesis, release and turn over rate in adult animals (Boadle-Biber et al., 1987; Tao and Auerbach, 1994). On the contrary, withdrawal to long-term exposure to morphine profoundly depressed serotonin level in many regions in CNS (Tao et al., 1998). Thus, withdrawal-induced reduction in brain serotonin level might be responsible for somatic as well as subjective symptoms of morphine withdraw. This idea is supported by the finding that administrating SERT inhibitor significantly attenuated the naloxone-precipitated hyperactivity of noradrenergic locus coeruleus (LC) neurons, an important brain substrate of opiate withdrawal, and the behavior changes (Akaoka and Aston-Jones, 1993; el-Kadi and Sharif, 1995; Harris and Aston-Jones, 2001; Lu et al., 2001; Rafieian-Kopaei et al., 1995). However, all these studies were performed in adult animals, yet no similar investigation is applied on the neonatal withdrawal syndrome. Thus, in the present study we determined whether naloxone-precipitated withdrawal syndrome in 5 day old rats could be attenuated by three different SERT inhibitors, fluoxetine, clomopiramine and citolopram.

2. Methods and Materials:

2.1 Chemicals

Morphine was purchased from the Narcotics Bureau of the National Health Adminstration, Taipei, Taiwan. Naloxone, fluoxetine, clomipramine, and citalopram were purchased from Tocris Cookson Ltd (U.K).

2.2 Animals.

Female Sprauge-Dawley rats (200 gm- 250 gm, purchased from National Experimental Animal Center, Taipei, Taiwan) were housed individually in plexiglass cages on a 12-hour light-dark cycle in Animal Center of Taipei Medical University. The room temperature was maintained at 24^{O}C. Food and water were available *ad libitum* throughout the experiment.

2.3 Animal model of prenatal- and post-natal exposure to morphine

Adult female rats received bi-daily subcutaneous injection of morphine (2 mg/kg) for seven days before mating. After conception, the dosage of morphine was increased by 1 mg/kg per week. After delivery of the newborn rats, the dosage of morphine was increased by 1 mg/kg till the offspring were 5 day old when they were used for experiment. Control dam rats received bi-daily injection of normal saline. Rats born to morphine-treated dam rats are denoted as the morphine group rats, and rats born to saline-treated dam rats are denoted as the control group rats.

2.4 Naloxone-induced behavioral changes in the neonatal rats

To precipitate the withdrawal syndrome in the neonatal rats, we subcutaneously injected 1 mg/kg of naloxone, a non-selective opioid receptor antagonist, into the morphine group rats on PND 5. We, then, quantified the frequency and latency of abdominal stretching and yawning during a 2-hour observation period as the index for the severity of morphine withdrawal syndrome.

2.5 Postnatal treatment of fluoxetin(Flu)e, citolopram(Cit) and clopromarine(Clm) on the neonatal rats

Morphine group rats will receive subcutaneous injection of Flu (10 mg/kg, 20 mg/kg, or 40 mg/kg), Cit (2 mg/kg, 5 mg/kg, or 10mg/kg) or Clm (2 mg/kg, 5 mg/kg, or 10 mg/kg) 30 minutes before injection of naloxone.

2.6 Statistic analysis

Ratios of rats presenting naloxone-precipitated abdominal stretching and Yawning were analyzed using Chi-square test. For other data, one way ANOVA with post hoc Newman-Keuls test was used

Result

3.1 The effect of fluoxetine, clomipramine and citalopram on the expression of naloxone-precipitated abdominal stretching

All the morphine group rats presented frequent abdominal stretching after injection with naloxone. On the contrary, none of the control rats had abdominal stretching after injection with naloxone. Pre-injection with one of SERT inhibitors reducing the ratio of rat presented abdominal stretching in a dose-dependent manner (Fig.1A). The EC_{50} for flueoxitine, clomipramine, and citalopram in inhibiting naloxone-precipitating abdominal stretching is roughly between 20-40 mg/kg, 5-10 mg/kg, and 5-10 mg/kg, respectively. In addition, the SERT inhibitors also significantly prolonged the latency and decreased the frequency of abdominal stretching in the morphine group rats (Fig 1B ,C).

3.2 The effect of fluoxetine, clomipramine and citalopram on the expression of naloxone-precipitated yawning

Similar to that of abdominal stretching, all the morphine group rats presented yawning, and none of the control rats had yawning after injection with naloxone. Obviously, all three SERT inhibitors are more effective in attenuating the presentation of yawning than in attenuating abdominal stretching since only 20 mg/kg fluoxetine could reduce the ratio of rats presenting yawning to only 23% and 40 mg/kg fluoxetine could reduce to only 7% (Fig 2). On the other hand, 2 mg/kg of clomipramine or citalopram could complete abolish the symptoms of yawning.

Discussion

The present study demonstrated that inhibitor of SERT could effectively abolished the morphine withdrawal syndrome elicited by naloxone on the neonatal rats chronically and passively exposed to morphine. The major morphine withdrawal syndromes measured in this study are abdominal stretching and yawn. Although both symptoms have been documented in adult rats (Chen et al., 2003; Ramabadran, 1983), the expression of yawning is not reported in the neonatal rats including our previous report (Tao et al., 2001; Yeh et al., 2002). The reason for the lack of this presentation in our previous study is not clear. Nevertheless, yawning is one of apparent symptom of morphine or heroin withdrawal found in human including newborn baby (Bickel et al., 1988; O'Brien, 1996; Ostrea et al., 1975).

There are two reasons for choosing the three SERT inhibitors in this study. The first is that they are all highly potent in inhibiting SERT, and are at least 100 fold less potent in inhibiting nor-epinephrine reuptake transporter (NET) or dopamine reuptake transporter (DAT). According to the ligand binding analysis, the potency for these three compounds to binding to SERT is proximally 0.75 nM for citalopram, 0.5 nM for clomiprmaine and 2 nM for fluoxetine, and their potency for binding to norepinephrine reuptake transporter is roughly 3000 nM for citalopram, 100-200 nM for clomipramine, and 500 nM for fluoxetine (Millan et al., 2001; Owens et al., 1997). Their inhibitory potencies on DAT are far less than that in inhibiting NET. The second is that they are all commonly used in clinics, mainly used for anti-depressant, and their safety and side effect have been well-documented.

The rank order of the potency for the three SERT inhibitors in attenuating abdominal stretching or yawning is seems to be citalopram＝clomipramine >fluoxetine, and the relative

ratio of EC_{50} in attenuating abdominal stretching between citalopram, clomipramine, and fluoxetine is roughly 1:1: 4 (5-10 mg/kg for citalopram, 5-10 mg/kg for clomipramine, 20-40 mg/kg for fluoxetine). This rank order and relative potency ratio is rather close to their potency in inhibiting SERT when measured by ligand binding analysis, which is 1:0.7: 2.8 as according to ligand binding analysis (0.75 nM for citalopram, 0.5 nM for clomiprmaine and 2 nM for fluoxetine) (Millan et al., 2001; Owens et al., 1997). Apparently, it is not consistent to that in inhibiting the NET, suggesting that the inhibitory effect of these three compounds in naloxone-precipitated withdrawal syndrome is likely through their inhibition on SERT. Previous reports had proved that serotonin system is one of the earliest neurotransmission system to be formed in the CNS (Lauder et al., 1982). At birth, this system is well formed both in structure and function and serotonin system contributes to the development of brain, especially for the cortex, in the early life (Pranzatelli, 1994; Pranzatelli and Martens, 1992). More importantly, the existence of SERT in the neonatal brain has been clearly demonstrated(McGrath et al., 1997), and application of SERT inhibitor, like clomipramine did altered the function of the serotonin-mediated neurotransmission (Hansen and Mikkelsen, 1998; Foguet et al., 1993). These reports further support our notion that these SERT inhibitors could act at SERT in neonatal rat brain.

It appears that the potent of all the examined SERT inhibitors in attenuating naloxone-precipitated yawning behavior are significant higher than that in abdominal stretching behavior. 20 mg/kg of fluoxetine could reduced the percentage of population presenting yawning to 23% but only reduced percentage of population presenting abdominal stretching to 84.6 %. On the other hand, 2 mg/kg of clompipramine or citalopram could completely abolish the yawning behaviors, but 10 mg/kg are required to abolish abdominal stretching. Such different sensitivity to SERT inhibitor suggests that the difference in underlying pathological mechanisms for these two different withdrawal symptoms. Previous report had indicated a complexity of neural systems in generating and modifying yawning behaviors (Argiolas and Melis, 1998). In brief, generation of yawning behavior includes activation of dopamine receptor, oxytocin receptor and glutamate receptor. On the contrary, activation of opioid receptor or serotonin receptor subtype, $5\text{-}HT_{1A}$ receptor or $5\text{-}HT_2$ receptor, serves as an inhibitory mechanism for yawn response. It is likely that theses neurophysiological properties render the sensitivity of SERT inhibitors to attenuate naloxone-precipitated yawning behavior by enhancing serotonin-mediated neurotransmission. However, the neural pathway for abdominal stretching is not known at present. We suspect that the ole of serotonin receptor in abdominal stretching might be not as essential as that in yawning behavior.

The result of this study is quite consistent with the study of SERT inhibitor on morphine withdrawal syndrome of adult rats (el-Kadi and Sharif, 1995; Lu et al., 2001; Rafieian-Kopaei et al., 1995), suggesting that either a deficiency in the serotonin-mediated neurotransmission contribute to the expression of morphine withdrawal syndrome both in adult and neonatal rats, or an increase in serotonin function by the action of SERT inhibitor counteracts the mechanisms responsible for the expression of morphine withdrawal syndrome. However, taking into account

the finding that NMDA receptor antagonists, like MK-801 or dextromethorphan, are as effective as SERT inhibitor in attenuating morphine withdrawal syndrome in both adult and neonatal rats (Tanganelli et al., 1991; Tiseo et al., 1994; Tokuyama et al., 1996; Trujillo and Akil, 1991; Yeh et al., 2002), it seems both glutamate and serotonin neurotransmitter systems are functionally converging in the neural network in generation of the withdrawal syndrome. It might be that the functions of these two systems are altered in opposite way during abstinence state, in which the function of NMDA receptor system is increased and the function of serotonin system is decrease. Alternatively, the functions of both systems might be not changed at all in abstinence state. Rather activation of NMDA receptor is required for the expression of withdrawal syndrome, and enhanced serotonin system in some way could counteract directly or indirectly with it. Both hypotheses all support the effectiveness of NMDA receptor antagonist and SERT inhibitor in attenuating morphine withdrawal syndrome.

In summary, the present study support the notion that SERT inhibitors is of potential in treating the acute morphine withdrawal syndrome in newborn baby. However, the pharmacokinetics, pharmacodynamics, as well as the drug safety of SERT inhibitors in newborn stage require carefully evaluation before any clinical application.

Acknowledge

This study is supported by a grant from National Science Council of Taiwan (NSC 92-2314-B-038-023)

Reference

Akaoka, H. and G. Aston-Jones, 1993, Indirect serotonergic agonists attenuate neuronal opiate withdrawal, Neuroscience 54, 561.

Archer, T., G. Jonsson, B.G. Minor and C. Post, 1986, Noradrenergic-serotonergic interactions and nociception in the rat, Eur J Pharmacol 120, 295.

Argiolas, A. and M.R. Melis, 1998, The neuropharmacology of yawning, Eur J Pharmacol 343, 1.

Bickel, W.K., M.L. Stitzer, I.A. Liebson and G.E. Bigelow, 1988, Acute physical dependence in man: effects of naloxone after brief morphine exposure, J Pharmacol Exp Ther 244, 126.

Boadle-Biber, M.C., J.N. Johannessen, N. Narasimhachari and T.H. Phan, 1987, Activation of cortical tryptophan hydroxylase by acute morphine treatment: blockade by 6-hydroxydopamine, Eur J Pharmacol 139, 193.

Brase, D.A., 1979, Roles of serotonin and gamma-aminobutyric acid in opioid effects, Adv Biochem Psychopharmacol 20, 409.

Chen, J.C., P.L. Tao, J.Y. Li, C.H. Wong and E.Y. Huang, 2003, Endomorphin-1 and -2 induce naloxone-precipitated withdrawal syndromes in rats, Peptides 24, 477.

el-Kadi, A.O. and S.I. Sharif, 1995, The role of 5-HT in the expression of morphine withdrawal in mice, Life Sci 57, 511.

Foguet, M., J.A. Hartikka, K. Schmuck and H. Lubbert, 1993, Long-term regulation of serotonergic activity in the rat brain via activation of protein kinase A, Embo J 12, 903.

Hansen, H.H. and J.D. Mikkelsen, 1998, Long-term effects on serotonin transporter mRNA expression of chronic neonatal exposure to a serotonin reuptake inhibitor, Eur J Pharmacol 352, 307.

Harris, G.C. and G. Aston-Jones, 2001, Augmented accumbal serotonin levels decrease the preference for a morphine associated environment during withdrawal, Neuropsychopharmacology 24, 75.

Jacobs, B.L. and E.C. Azmitia, 1992, Structure and function of the brain serotonin system, Physiol Rev 72, 165.

Lauder, J.M., J.A. Wallace, H. Krebs, P. Petrusz and K. McCarthy, 1982, In vivo and in vitro development of serotonergic neurons, Brain Res Bull 9, 605.

Lu, L., W.J. Su, W. Yue, X. Ge, F. Su, G. Pei and L. Ma, 2001, Attenuation of morphine dependence and withdrawal in rats by venlafaxine, a serotonin and noradrenaline reuptake inhibitor, Life Sci 69, 37.

McGrath, K.E., F.J. Seidler and T.A. Slotkin, 1997, Convergent control of serotonin transporter expression by glucocorticoids and cocaine in fetal and neonatal rat brain, Brain Res Dev Brain Res 104, 209.

Millan, M.J., A. Gobert, F. Lejeune, A. Newman-Tancredi, J.M. Rivet, A. Auclair and J.L. Peglion, 2001, S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine, J Pharmacol Exp Ther 298, 565.

Murphy, D.L., 1990, Neuropsychiatric disorders and the multiple human brain serotonin receptor subtypes and subsystems, Neuropsychopharmacology 3, 457.

O'Brien, C.P., 1996, Drug application and drug abuse, in: The Goodman and Gilman's The Pharmacological Basis of Therapeutics, ed. J.G. Hardman, Limbrid,L.E.,Molinoff, P.B., Ruddon, R.W., Gilman, A.G. (McGraw Hill, New York) p. 557.

Ostrea, E.M., Jr., C.J. Chavez and M.E. Strauss, 1975, A study of factors that influence the severity of neonatal narcotic withdrawal, Addict Dis 2, 187.

Owens, M.J., W.N. Morgan, S.J. Plott and C.B. Nemeroff, 1997, Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites, J Pharmacol Exp Ther 283, 1305.

Pranzatelli, M.R., 1994, Dissociation of the plasticity of 5-HT1A sites and 5-HT transporter sites, Neurochem Res 19, 311.

Pranzatelli, M.R. and J.M. Martens, 1992, Plasticity and ontogeny of the central 5-HT transporter: effect of neonatal 5,7-dihydroxytryptamine lesions in the rat, Brain Res Dev Brain Res 70, 191.

Rafieian-Kopaei, M., A.M. Gray, P.S. Spencer and R.D. Sewell, 1995, Contrasting actions of acute or chronic paroxetine and fluvoxamine on morphine withdrawal-induced place conditioning, Eur J Pharmacol 275, 185.

Ramabadran, K., 1983, Naloxone-precipitated abstinence in mice, rats and gerbils acutely dependent on morphine, Life Sci 33, 385.

Singh, V.P., N.K. Jain and S.K. Kulkarni, 2001, On the antinociceptive effect of fluoxetine, a selective serotonin reuptake inhibitor, Brain Res 915, 218.

Tanganelli, S., T. Antonelli, M. Morari, C. Bianchi and L. Beani, 1991, Glutamate antagonists prevent morphine withdrawal in mice and guinea pigs, Neurosci Lett 122, 270.

Tao, P.L., G.C. Yeh, C.H. Su and Y.H. Wu, 2001, Co-administration of dextromethorphan during pregnancy and throughout lactation significantly decreases the adverse effects associated with chronic morphine administration in rat offspring, Life Sci 69, 2439.

Tao, R. and S.B. Auerbach, 1994, Increased extracellular serotonin in rat brain after systemic or intraraphe administration of morphine, J Neurochem 63, 517.

Tao, R., Z. Ma and S.B. Auerbach, 1998, Alteration in regulation of serotonin release in rat dorsal raphe nucleus after prolonged exposure to morphine, J Pharmacol Exp Ther 286, 481.

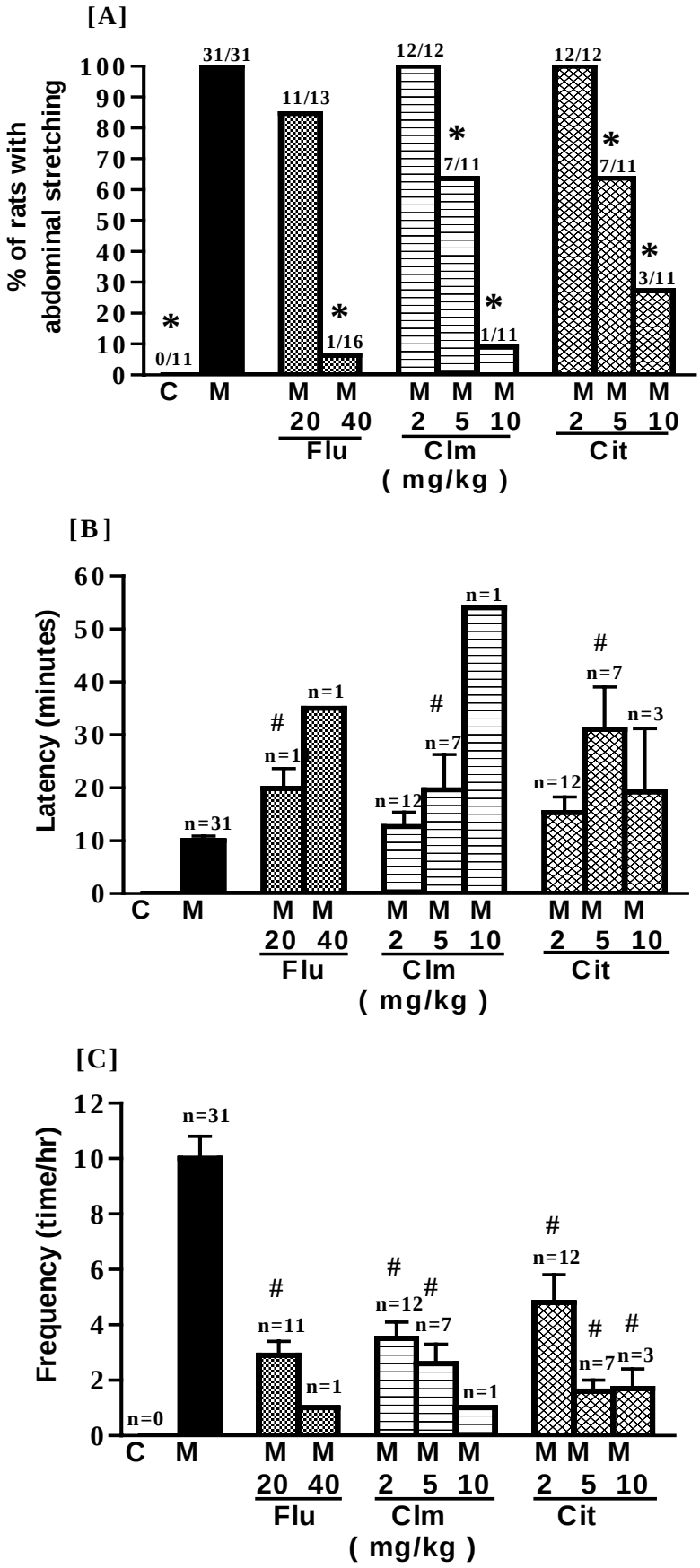
Tiseo, P.J., J. Cheng, G.W. Pasternak and C.E. Inturrisi, 1994, Modulation of morphine tolerance by the competitive N-methyl-D- aspartate receptor antagonist LY274614: assessment of opioid receptor changes, J Pharmacol Exp Ther 268, 195.

Tokuyama, S., H. Wakabayashi and I.K. Ho, 1996, Direct evidence for a role of glutamate in the expression of the opioid withdrawal syndrome, Eur J Pharmacol 295, 123.

Trujillo, K.A. and H. Akil, 1991, Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801, Science 251, 85.

Volpe, J.J., 1995, Teratogenic effects of drugs and passive addiction, in: Neurology of the Newborn, ed. J.J. Volpe (W.B. Saunder Company, p. 811.

Yeh, G.C., P.L. Tao, J.Y. Chen, M.C. Lai, F.S. Gao and C.L. Hu, 2002, Dextromethorphan attenuates morphine withdrawal syndrome in neonatal rats passively exposed to morphine, Eur J Pharmacol 453, 197.


[A]
% of rats with abdominal stretching
100
90
80
70
60
50
40
30
20
10
0
0/11
31/31
11/13
1/16
12/12
7/11
1/11
12/12
7/11
3/11
*
C M M M M M M M M M
20 40 2 5 10 2 5 10
Flu Clm Cit
(mg/kg)
[B]
Latency (minutes)
60
50
40
30
20
10
0
n=31
n=1
n=1
n=12
n=7
n=1
n=12
n=7
n=3
#
C M M M M M M M M M
20 40 2 5 10 2 5 10
Flu Clm Cit
(mg/kg)
[C]
Frequency (time/hr)
12
10
8
6
4
2
0
n=0
n=31
n=11
n=1
n=12
n=7
n=1
n=12
n=7
n=3
#
C M M M M M M M M M
20 40 2 5 10 2 5 10
Flu Clm Cit
(mg/kg)

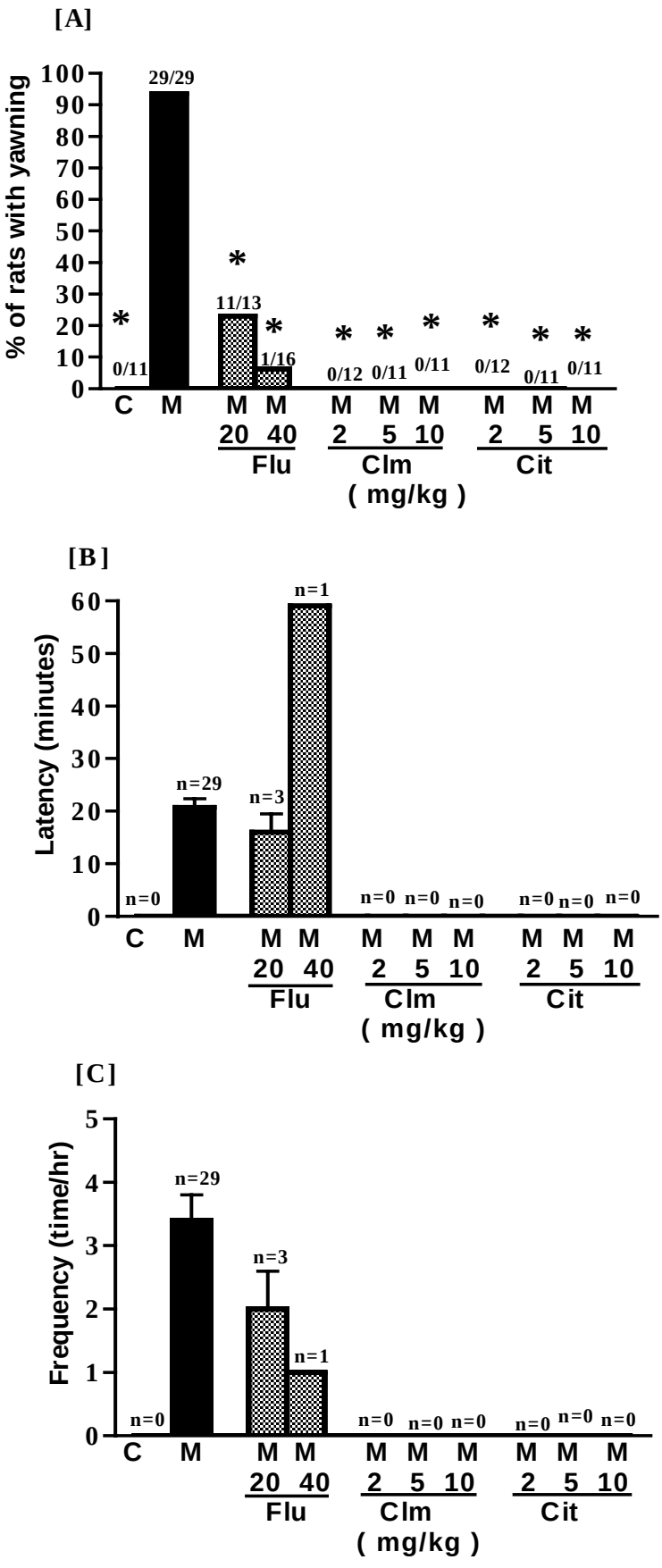

[A]
% of rats with yawning
100
90
80
70
60
50
40
30
20
10
0
29/29
11/13
1/16
0/11
0/12
0/11
0/11
0/12
0/11
0/11
*
C
M
M 20
M 40
Flu
M 2
M 5
M 10
Clm
(mg/kg)
M 2
M 5
M 10
Cit
[B]
Latency (minutes)
60
50
40
30
20
10
0
n=1
n=29
n=3
n=0
C
M
Flu
Clm
(mg/kg)
Cit
[C]
Frequency (time/hr)
5
4
3
2
1
0
n=29
n=3
n=1
n=0

Figure Legends

Figure 1. The effect of fluoxetine (Flu), clomipramine (Clm) and citalopram (Cit) on the naloxone-precipitated abdominal; stretching of control (C) and morphine (M) group rats. Data are mean ± S.E. [A]Percentage of rats presenting abdominal stretching in response to naloxone injection (1 mg/kg s.c.). The responding ratio of each experimental group is listed on the top pf each bar. [B]Latency to the first abdominal stretching. The number of rats showing abdominal stretching (n) are listed on the top of each bar. Statistic analysis was not able to performed when the number is lower than 3. A similar condition also occurred when comparing the frequency of abdominal stretching in [C].

* mean significantly different from that of morphine group rats (M) ($p < 0.05$, Chi-square test).

mean significantly different from that of morphine group rats (M) ($P < 0.05$, one-way ANOVA with Newman-Keuls test)

Figure 2. The effect of fluoxetine (Flu), clomipramine (Clm) and citalopram (Cit) on the naloxone-precipitated yawning of control (C) and morphine (M) group rats. Data are mean ± S.E. [A]Percentage of rats presenting yawning in response to naloxone injection (1 mg/kg s.c.). The responding ratio of each experimental group is listed on the top pf each bar. [B]Latency to the first yawning. The number of rats showing yawning (n) are listed on the top of each bar. Statistic analysis was not able to performed when the number is lower than 3. A similar condition also occurred when comparing the frequency of yawning in [C].

* mean significantly different from that of morphine group rats (M) ($p < 0.05$, Chi-square test).

計劃成果使用

已投寄 European Journal of Pharmacology　正審查中