This article was downloaded by:
On: *22 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Antidepressant-like effects of piperine and its derivative, antiepilepsirine

Song Li[a]; Che Wang[ab]; Wei Li[c]; Kazuo Koike[c]; Tamatsu Nikaido[c]; Min -Wei Wang[b]
[a] Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang, China [b] China-Japan Research Institute of Medical and Pharmaceutical Sciences, Shenyang Pharmaceutical University, Shenyang, China [c] Faculty of Pharmaceutical Sciences, Toho University, Chiba, Japan

Online publication date: 27 July 2010

To cite this Article Li, Song , Wang, Che , Li, Wei , Koike, Kazuo , Nikaido, Tamatsu and Wang, Min -Wei(2007) 'Antidepressant-like effects of piperine and its derivative, antiepilepsirine', Journal of Asian Natural Products Research, 9: 5, 421 — 430

To link to this Article: DOI: 10.1080/10286020500384302

URL: http://dx.doi.org/10.1080/10286020500384302

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Asian Natural Products Research, Vol. 9, No. 5, July–August 2007, 421–430

Taylor & Francis
Taylor & Francis Group

Downloaded At: 19:07 22 January 2011

Antidepressant-like effects of piperine and its derivative, antiepilepsirine

SONG LI†, CHE WANG†‡, WEI LI¶, KAZUO KOIKE¶, TAMATSU NIKAIDO¶ and MIN -WEI WANG†‡*

†Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, China
‡China-Japan Research Institute of Medical and Pharmaceutical Sciences, Shenyang Pharmaceutical University, Shenyang 110016, China
¶Faculty of Pharmaceutical Sciences, Toho University, Miyama 2-2-1 Funabashi, Chiba 274-8510, Japan

(Received 20 April 2005; revised 15 June 2005; in final form 16 June 2005)

In the present study, antidepressant-like effects of piperine (PIP) and its derivative, antiepilepsirine (AES), were investigated in two depressive models: forced swimming test (FST) and tail suspension test (TST). To further explore the mechanisms underlying their antidepressant-like activities, the brain monoamine levels and monoamine oxidase A and B (MAO-A and MAO-B) activities were also determined. The research results for the first time indicated that after two weeks of chronic administration, PIP and AES at doses of 10–20 mg/kg significantly reduced the duration of immobility in both FST and TST, without accompanying changes in locomotor activity in the open-field test. But at the dose of 80 mg/kg, the antidepressant activity of both PIP and AES returned to the control level in the TST and FST. In the monoamine assay, chronic AES administration significantly elevated the dopamine level in striatum, hypothalamus and hippocampus, and also increased the serotonin level in the hypothalamus and hippocampus. In contrast, chronic treatment of PIP only enhanced the serotonin level in the hypothalamus and hippocampus but did not influence the dopamine level. Moreover, both PIP and AES showed no effects on level of noradrenaline in these brain regions. The MAO activity assay also indicated that PIP and AES showed a minor MAO inhibitory activity. In the present study, we demonstrated that the antidepressant-like effects of PIP and AES might depend on the augmentation of the neurotransmitter synthesis or the reduction of the neurotransmitter reuptake. Antidepressant properties of PIP were supposed to be mediated via the regulation of serotonergic system, whereas the mechanisms of antidepressant action of AES might be due to its dual regulation of both serotonergic and dopaminergic systems.

Keywords: Piperine; Antiepilepsirine; Antidepressant; Monoamine; MAO activity

1. Introduction

Depression is a serious mood disorder that affects 17–20% of the population of the world and may result in major social and economic consequences [1]. Significant progress has been made in the research works for treatment of depression to make this common disease more treatable, however, the therapeutic response requires several weeks or months of treatment,

*Corresponding author. Email: wangmw_spu@yahoo.com.cn

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500384302

and contemporary antidepressants can produce many side effects. Moreover, some patients do not have responses to the currently available antidepressants [2,3]. In Oriental society, herbal preparations are widely used by consumers and have a long history of use as medicines. Some of them may be effective alternatives in the treatment of depression, as in the case of St. John's wort, a Western herb [4].

The first effective antidepressants, such as monoamine oxidase inhibitors and tricyclic antidepressants, augmented serotonin and noradrenaline levels in the synapse [5]. Recent reports showed that the dopaminergic system also had important roles in the pathophysiology of depression. The serotonergic system has long been implicated in the pathogenesis of depression. Some of the most compelling evidence involves the alleviation of depression caused by serotonin selective reuptake inhibitors (SSRIs), which increase the availability of serotonin at the synapse [6]. Studies of tryptophan depletion also confirmed the relationship between serotonin and depression [7]. Noradrenaline is also found throughout the brain, and its functions include acting as a general regulator of mood and response to stimuli such as stress [8]. Depression seems to be associated with a hypofunction of the noradrenergic system, and some antidepressants act by increasing the synaptic availability of norepinephrine [9]. Moreover, increasing evidence from human and animal studies suggested the relationship between dopamine transmission and depression in the central nervous system. In depressed patients, a compensatory up-regulation of D_2 receptor density was observed in the basal ganglia/cerebellum in comparison with healthy subjects, consistent with the hypothesis of an association between depression and deficiency of dopamine transmission [10]. The animal models of depression also suggest an implication of dopamine in the physiopathology of depression [11,12].

Piperine (PIP), a constituent isolated from black pepper (*Piper nigrum* Linn.) or long pepper (*Piper longum* Linn.), belongs to the chemical family of Cinnamamides, which have been reported to process sedative, hypnotic, anticonvulsant and muscle relaxant actions. Antiepilepsirine (AES), one derivative of PIP, has been used clinically as an efficacious anticonvulsant, exhibiting a more potent effect than PIP. To our knowledge, no data are currently available about the behavioural effect in depressed animals after consecutive oral exposure with PIP and AES. The aims of our present study will examine the therapeutic effects of PIP and AES on depressive behaviours in the forced swimming test and the tail suspension test in mice, and determine whether the alteration of monoamine levels and monoamine oxidase A (MAO) activities might predict the antidepressant properties of PIP and AES.

2. Results and discussion

2.1 *Effects of PIP and AES on the duration of immobility in TST and FST in mice*

In this study, we examined the antidepressant effects of PIP and one of its derivatives, AES (figure 1), in two behavioural tests, forced swimming test (FST) and tail suspension test (TST). The decreased immobility duration in animals of TST and FST may predict the efficacy of antidepressants [13,14], as they are sensitive and selective for clinically used antidepressant drugs. PIP and AES, at doses of 10–20 mg/kg significantly decreased the immobility time in the animal models (tables 1 and 2), and their antidepressant effects at dose of 10 mg/kg seemed to be more potent than that of 20 mg/kg. Furthermore, at higher dose of PIP and AES (80 mg/kg), the duration of immobility was returned to the control level.

Downloaded At: 19:07 22 January 2011

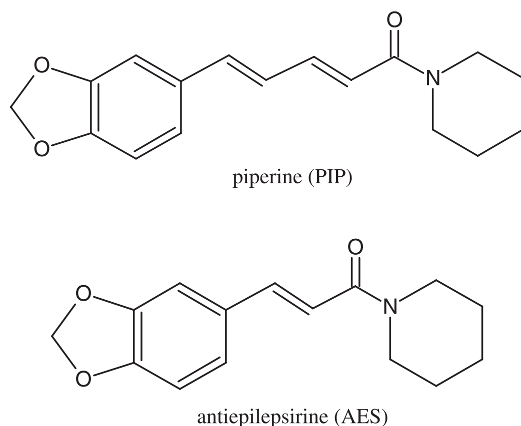

Figure 1. Chemical structures of PIP and AES.

Downloaded At: 19:07 22 January 2011

The reason for this conversed dose responses curve might be due to their sedative and muscle relaxation activities [15,16]. As expected, the reference antidepressant fluoxetine at the dose of 20 mg/kg significantly reduced immobility time in this animal model. These results suggested for the first time that PIP and AES might cause an antidepressant-like effect in a certain dose range.

2.2 *Effects of PIP and AES on open-field behaviour test in mice*

In the present study, PIP and AES at doses of 10–20 mg/kg, and fluoxetine at a dose of 20 mg/kg, significantly reduced immobility time in the TST and FST. However, it was also possible that the ability of PIP and AES to decrease the immobility in the FST and TST might

Table 1. Effects of PIP, AES and fluoxetine on the duration of immobility in TST in mice (mean ± SEM, $n = 10$).

		Duration of immobility (s)	
Drug	*Dose (mg/kg)*	*Week 1*	*Week 2*
Control		71.3 ± 4.1	68.2 ± 4.8
PIP	20	58.4 ± 2.1*	46.7 ± 3.2*
	10	47.6 ± 2.2*	37.9 ± 1.8*
AES	20	57.6 ± 2.7*	49.2 ± 1.6*
	10	49.3 ± 1.2*	38.9 ± 2.7*
Fluoxetine	20	29.6 ± 4.3*	27.2 ± 2.9*

*$P < 0.01$ compared with control group.

Table 2. Effects of PIP, AES and fluoxetine on the duration of immobility in FST in mice (mean ± SEM, $n = 10$).

		Duration of immobility (s)	
Drug	*Dose (mg/kg)*	*Week 1*	*Week 2*
Control		74.1 ± 4.4	66.6 ± 3.7
PIP	20	51.2 ± 3.7*	50.1 ± 3.3*
	10	41.2 ± 2.9*	35.8 ± 2.3*
AES	20	58.9 ± 3.7*	44.7 ± 1.7*
	10	39.9 ± 4.2*	32.4 ± 2.9*
Fluoxetine	20	31.1 ± 4.0*	26.8 ± 2.6*

*$P < 0.01$, when compared with control group.

Table 3. Effects of PIP, AES and fluoxetine on the locomotor activity in open field test in mice (mean ± SEM, $n = 10$).

		Locomotor activity		
Drug	*Dose (mg/kg)*	*Ambulation*	*Rearing*	*Grooming*
Control		82.5 ± 8.5	25.4 ± 3.2	3.6 ± 1.1
PIP	80	47.8 ± 4.5**	12.6 ± 2.1**	2.2 ± 0.7*
	20	76.4 ± 7.1	21.6 ± 3.3	4.1 ± 1.4
	10	89.2 ± 8.3	24.9 ± 2.7	3.7 ± 0.9
AES	80	54.1 ± 3.9**	11.9 ± 1.8**	2.5 ± 1.2*
	20	69.8 ± 7.2	20.5 ± 2.2	4.5 ± 1.8
	10	84.2 ± 9.1	29.3 ± 4.1	3.7 ± 1.5
Fluoxetine	20	88.5 ± 6.9	27.4 ± 2.5	3.5 ± 1.3

$*P < 0.05$, $**P < 0.01$ compared with control group.

Downloaded At: 19:07 22 January 2011

be simply dependent on the enhancement of spontaneous motor activity. In our present study, the open field test indicated that PIP and AES at the dose of 10–20 mg/kg did not influence the spontaneous motor activity and at a dose of 80 mg/kg even showed sedative effects (table 3). Thus, the PIP- and AES-induced decline in the immobility seemed not to be mediated by stimulation of the overall motor activity of the animals.

2.3 Effects of PIP and AES on the levels of monoamine neurotransmitter in different brain regions of mice

Much remains unclear about the neuropharmacology of depression; however, it is well known that an enhancement of neurotransmission of serotonin (5-hydroxytryptamine; 5-HT), norepinephrine (NE), or both, underlies the antidepressant response associated with most agents presently available to treat major depression [17]. A recent review on the relationship between dopamine and depression suggested that the dopaminergic system is another appropriate target for antidepressant drugs [18]. Consequently, each of these systems has been the target for drug development efforts. Most currently available antidepressants exert their effects predominantly on one monoaminergic system, although it is unlikely that pharmacological manipulation of a single neurotransmitter in relative isolation would produce changes sufficient to remedy severe neurochemical dysfunction. Indeed, there is abundant evidence from anatomical, electrophysiological and pharmacological studies that the interactions between neurotransmitter systems are important [19–21]. Anatomical studies have shown that dopamine (DA) and 5-HT systems project to common terminal fields in the prefrontal cortex (PFC), striatum (STR), and nucleus accumbens (NACC). Microdialysis studies have shown that the enhanced level of the terminal 5-HT can facilitate DA release [20]. In light of these data, patients with depressive disorders may benefit most from a drug remedy with a very broad spectrum of neurochemical effects.

To further investigate the mechanisms of PIP and AES on anti-depressant actions, the effects of PIP, AES and fluoxetine on levels of 5-HT, 5-hydroxyindole acetic acid (5-HIAA), NE, 3-methoxy-4-hydroxyphenylglycol (MHPG), and DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured. As shown in tables 4–6, both PIP and AES at dose of 10 mg/kg significantly elevated the total amount of 5-HT and 5-HIAA in hypothalamus and hippocampus of mouse brain. The ratio of 5-HIAA/5-HT (table 7), a major index of 5-HT turnover, was decreased lightly after chronic administration of PIP and AES.

Table 4. Effects of PIP and AES on the concentrations of NE and MHPG in the mouse brain.

Region	*Group*	*NE*	*MHPG*	*NE + MHPG*
Hippocampus	Control	678.12 ± 31.43	12.58 ± 1.02	690.70 ± 32.45
	PIP (10 mg/kg)	708.46 ± 27.01	11.24 ± 0.79	719.70 ± 27.80
	AES (10 mg/kg)	686.31 ± 29.66	13.72 ± 1.01	700.03 ± 30.67
	Fluoxetine	795.17 ± 23.19	14.70 ± 0.96	809.87 ± 24.15
Frontal cortex	Control	607.35 ± 30.12	26.17 ± 2.67	633.52 ± 32.79
	PIP (10 mg/kg)	621.31 ± 27.21	23.98 ± 2.11	645.29 ± 29.32
	AES (10 mg/kg)	609.44 ± 31.03	21.06 ± 1.96	630.50 ± 32.99
	Fluoxetine	775.58 ± 24.19*	29.70 ± 2.36	805.28 ± 26.55*
Hypothalamus	Control	2123.08 ± 106.78	42.12 ± 4.01	2165.2 ± 110.79
	PIP (10 mg/kg)	1998.56 ± 99.94	37.63 ± 3.93	2036.19 ± 103.87
	AES (10 mg/kg)	2097.33 ± 101.45	41.03 ± 2.97	2138.36 ± 104.42
	Fluoxetine	2501.37 ± 104.41	48.83 ± 3.66	2550.2 ± 108.07

Mice were sacrificed 60 min after the last administration. Concentrations are expressed as ng per g fresh weight of brain tissue. Data expressed as mean ± SEM ($n = 9$–10).
$*P < 0.05$ compared with control group.

Downloaded At: 19:07 22 January 2011

Table 5. Effects of PIP and AES on the concentrations of 5-HT and 5-HIAA in the mouse brain.

Region	*Group*	*5-HT*	*5-HIAA*	*5-HT + 5-HIAA*
Hippocampus	Control	662.87 ± 45.53	712.86 ± 48.31	1375.73 ± 93.84
	PIP (10 mg/kg)	812.37 ± 41.79**	766.32 ± 57.36	1578.69 ± 99.15**
	AES (10 mg/kg)	828.39 ± 37.61**	748.18 ± 40.11	1576.57 ± 77.72**
	Fluoxetine	858.41 ± 33.13**	734.19 ± 25.58	1592.6 ± 58.71**
Hypothalamus	Control	2477.40 ± 198.56	1957.96 ± 202.10	4435.36 ± 400.66
	PIP (10 mg/kg)	2980.22 ± 257.37*	2093.71 ± 155.50	5073.93 ± 412.87*
	AES (10 mg/kg)	3109.46 ± 191.53**	2034.25 ± 192.61	5143.71 ± 384.14**
	Fluoxetine	3508.29 ± 268.56**	2158.34 ± 184.31	5666.63 ± 452.87**

Mice were sacrificed 60 min after the last administration. Concentrations are expressed as ng per g fresh weight of brain tissue. Data expressed as mean ± SEM ($n = 9$–10).
$*P < 0.05$, $**P < 0.01$ compared with control group.

AES but not PIP at the dose of 10 mg/kg increased the total amount of DA, DOPAC and HVA in striatum, hippocampus and hypothalamus, whereas the ratio of DOPAC/DA and HVA/DA was also depressed (table 7). Moreover, both PIP and AES did not influence the levels of NE and its metabolites in the mouse brain (tables 4 and 7). Fluoxetine, the reference control in this study, enhanced the concentrations of extracellular serotonin by inhibition of its reuptake, also enhanced extracellular noradrenaline in the frontal cortex and extracellular dopamine in hypothalamus, hippocampus and striatum. The data accorded with the reports of Hughes *et al.* and Pozzi *et al.*, respectively [22,23]. All these results indicated that the effect of PIP on depression might be mediated via enhancement of 5-HT synthesis or inhibition of reuptake course, while the antidepressant property of AES was supposed to be related to regulations of both 5-HT and DA systems. The influence of these two compounds on the monoamine levels seemed to be different from that of fluoxetine, one of the selective serotonin reuptake inhibitors (SSRI).

2.4 MAO inhibitory activities of piperine, AES and fluoxetine

The effects of PIP, AES and fluoxetine after chronic administration on the MAO-A and MAO-B activities in mouse whole brain are shown in table 8. Oral administration of PIP and AES, at the doses of 10–20 mg/kg, inhibited MAO-A and MAO-B activities. Fluoxetine

Downloaded At: 19:07 22 January 2011

Table 6. Effects of PIP and AES on the concentrations of DA, DOPAC and HVA in the mouse brain.

Region	*Group*	*DA*	*DOPAC*	*HVA*	*DA + DOPAC + HVA*
Hippocampus	Control	53.72 ± 6.56	13.21 ± 0.88	52.19 ± 6.96	119.12 ± 14.4
	PIP (10 mg/kg)	51.17 ± 4.52	15.16 ± 1.01	49.93 ± 7.26	116.26 ± 12.79
	AES (10 mg/kg)	89.29 ± 6.08**	14.99 ± 0.52	55.95 ± 5.21	160.23 ± 11.81**
	Fluoxetine	75.35 ± 6.16*	12.47 ± 0.73	50.88 ± 7.01	138.7 ± 13.90*
Striatum	Control	21457.34 ± 1252.73	2699.79 ± 157.26	1643.33 ± 122.26	25800.46 ± 1532.25
	PIP (10 mg/kg)	23321.71 ± 1138.92	2478.97 ± 146.92	1597.26 ± 145.23	27397.94 ± 1431.07
	AES (10 mg/kg)	31840.85 ± 1578.33**	2902.21 ± 203.01	1806.34 ± 156.54	36549.40 ± 1937.88**
	Fluoxetine	30215.75 ± 1089.80**	2577.79 ± 177.23	1579.38 ± 139.66	34372.92 ± 1406.69*
Hypothalamus	Control	1000.34 ± 92.83	343.91 ± 28.83	302.12 ± 30.21	1646.37 ± 151.87
	PIP (10 mg/kg)	1044.52 ± 78.19	411.52 ± 45.34	344.31 ± 29.32	1800.35 ± 152.85
	AES (10 mg/kg)	1666.38 ± 101.25**	389.21 ± 47.34	353.71 ± 38.57	2409.30 ± 187.16**
	Fluoxetine	1525.83 ± 104.57*	358.57 ± 32.39	321.26 ± 32.33	2205.66 ± 169.29*

Mice were sacrificed 30 min after the last administration. Concentrations are expressed as ng per g fresh weight of brain tissue. Data expressed as mean ± SEM ($n = 9$–10).
$*P < 0.05$, $**P < 0.01$ compared with control group.

Table 7. Effects of chronic PIP and AES treatment on MHPG/NE, 5-HIAA/5-HT, DOPAC/DA and HVA/DA in different regions of mouse brain.

Region	*Group*	*MHPG/NE*	*5-HIAA/5-HT*	*DOPAC/DA*	*HVA/DA*
Hippocampus	Control	0.019 ± 0.003	1.075 ± 0.15	0.246 ± 0.03	0.972 ± 0.2
	PIP (10 mg/kg)	0.016 ± 0.002	0.943 ± 0.11	0.296 ± 0.02	0.976 ± 0.14
	AES (10 mg/kg)	0.02 ± 0.001	0.903 ± 0.09*	0.168 ± 0.06**	0.627 ± 0.11**
	Fluoxetine	0.018 ± 0.002	0.855 ± 0.1*	0.165 ± 0.03**	0.675 ± 0.13**
Frontal cortex	Control	0.043 ± 0.006			
	PIP (10 mg/kg)	0.039 ± 0.003			
	AES (10 mg/kg)	0.035 ± 0.005			
	Fluoxetine	0.038 ± 0.002			
Hypothalamus	Control	0.02 ± 0.002	0.79 ± 0.06	0.343 ± 0.06	0.302 ± 0.06
	PIP (10 mg/kg)	0.019 ± 0.003	0.703 ± 0.08	0.394 ± 0.06	0.33 ± 0.05
	AES (10 mg/kg)	0.02 ± 0.002	0.654 ± 0.13*	0.233 ± 0.03**	0.212 ± 0.03**
	Fluoxetine	0.02 ± 0.001	0.615 ± 0.14*	0.235 ± 0.05**	0.21 ± 0.04**
Striatum	Control			0.126 ± 0.02	0.077 ± 0.016
	PIP (10 mg/kg)			0.106 ± 0.01	0.068 ± 0.01
	AES (10 mg/kg)			0.091 ± 0.03**	0.057 ± 0.011**
	Fluoxetine			0.085 ± 0.02**	0.052 ± 0.008**

Data expressed as mean ± SEM ($n = 9$–10).
$*P < 0.05$, $**P < 0.01$, compared with control group.

Downloaded At: 19:07 22 January 2011

Table 8. Effects of chronic PIP and AES treatment on MAO activity in mouse brain.

	MAO activity (U/g protein)	
	MAO-A	*MAO-B*
Control	14.73 ± 3.14	15.98 ± 3.02
PIP 10 mg/kg	11.25 ± 2.18*	11.37 ± 2.29*
PIP 20 mg/kg	9.98 ± 2.54*	10.06 ± 3.11*
AES 10 mg/kg	10.37 ± 3.18*	11.56 ± 3.25*
AES 20 mg/kg	9.02 ± 3.13*	9.19 ± 1.92*
Fluoxetine	13.57 ± 1.93	13.95 ± 2.67

Data expressed as mean ± SEM ($n = 9$–10).
$*P < 0.05$ compared with control group.

(20 mg/kg) did not alter the activities of MAO-A and MAO-B in the same assay. MAO is the major catabolic enzyme of monoamines. Some MAO-A inhibitors are efficacious for treating depression while the inhibitors of MAO-B appear to be effective in preventing and treating Parkinson's disease. Kong *et al.* indicated that PIP inhibited the activity of MAO with an IC_{50} of 49.3 and 91.3 μmol/L, respectively for MAO-A and MAO-B subtypes [24]. Consistent with that report, both PIP and AES showed a faint MAO inhibitory activity in our study. But according to the present results, it was difficult to draw such a conclusion that MAO inhibition was the major mechanism contributing to the antidepressant activities of PIP and AES, because no significant changes occurred in the NE system. So synthesis enhancement or reuptake inhibition of serotonin or dopamine induced by administration of PIP and AES might be the major mechanism underlying their antidepressant activity, and played a more important role than MAO inhibition did.

In summary, chronic administration of PIP and AES not only decreased the immobility time in both FST and TST, but also augmented depression-related monoamine levels in different brain regions of mice, suggesting that antidepressant properties of PIP and AES were related to the serotonergic and dopaminergic mechanisms. This report could thus

be of interest in the study of the potential therapeutic application of PIP and its derivatives in the treatment of depression.

3. Materials and methods

3.1 Animals and treatment

Male ICR mice (35–37 g, 8 weeks of age) were used in this study. The animals were housed at 23 ± 1°C with a regular 12/12-h light/dark cycle (lights on from 07:30 to 19:30 h), and given standard food and water *ad libitum*. All mice were allowed at least 1 week to acclimatise to their housing environment before each experiment. During the habituation period, animals were handled once daily. All animal procedures were approved by the Shenyang Pharmaceutical University Animal Welfare Committee and conducted in accordance with the guidelines of the China Council on Animal Care. Drugs of various concentrations or vehicles were given orally once daily with minimum stimulus at 09:00–10:00 for 2 weeks. The behaviour tests were performed weekly 1 h after the last administration. For the neurotransmitter assay, the additional groups of animals were sacrificed by decapitation after 2 weeks' administration, the brains were quickly removed, and different brain regions (hippocampus, hypothalamus, striatum, frontal cortex) were dissected and washed in cold 0.9% saline and stored at −80°C until assay.

Downloaded At: 19:07 22 January 2011

3.2 Chemicals

PIP and AES with a purity of 99.8% were obtained as described in previous study [25], and dissolved in 0.9% saline after being dispersed with Tween-80. The final concentration of Tween-80 was less than 0.1%. The selective serotonin reuptake inhibitor fluoxetine was selected as reference control. Drugs or vehicle were administered in a volume of 10 ml/kg. Norepinephrine (NE), dopamine (DA), 5-hydroxytryptophan (5-HT), 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindolacetic acid (5-HIAA) were purchased from Sigma (St. Louis, MO, USA). These chemicals were dissolved in double-distilled water and were frozen at −80°C immediately until use. Fluoxetine and β-phenylethylamine (β-PEA) were purchased from Sigma (St. Louis, MO, USA).

3.3 Tail suspension test

The tail suspension test was based on the method of Steru [13]. Briefly, the mouse was individually suspended to the shelf by the tail with an adhesive tape (1 cm from the tip of tail) for 6 min with the head 80 cm to the floor. The test was carried out in a darkened room with minimal background noise. Mice were considered immobile only when they hung passively and completely motionless. The duration of immobility was recorded during the final 4 min of the test.

3.4 Forced swimming test

FST is a method to estimate the behavioural despair in stressful and inescapable situations [14]. The mouse was placed for 6 min in the glass cylinder (18 cm in diameter, 25 cm high)

filled with water at 23 ± 1°C to the height of 15 cm. The time of immobility (passive floating, when the animal was motionless or doing only slight movements with tail or one hind limb) was measured during the last 4 min of the test.

3.5 Open field test

The studies were performed in mice according to a slightly modified method of Archer [26]. The open-field apparatus consisted of a circular base (80 cm in diameter, 20 cm high wall) having three concentric circles of 14, 28 and 42 cm radius, divided into 36 units without walls. The mouse was placed individually in the centre of the arena and allowed to explore freely. The ambulation, rearing and numbers of grooming were recorded for 3 min. Each mouse was tested individually and only once.

3.6 Chemical assay

NE, DA, 5-HT, and their metabolites (MHPG, DOPAC, HVA, 5-HIAA) were determined by high performance liquid chromatography (HPLC, Shimadzu, LC-6A, Tokyo, Japan) with electrochemistry detector (ECD, BAS Amperometric Detector, CC-4, USA) systems. A reverse-phase column (Dikma, diamond, C-18 ODS, 250 × 4 mm, USA) was used for separation. The working electrode potential of the detector was set at 760 mV. The composition of the mobile phase was 0.1 mol/L acetate-citrate buffer at pH 3.7, containing 15% methanol, 1.09 mmol/L octyl sodium sulphate acid, 0.4 mmol/L dibutylamine, and 0.2 mmol/L EDTA. The flow rate was 1.2 ml/min.

Downloaded At: 19:07 22 January 2011

3.7 Monoamine neurotransmitter determination

The 5-HT, NE, DA and their metabolites levels were determined simultaneously in mouse different brain regions by a modification of methods [27]. Briefly, animals were decapitated 60 min later after the last administration of 2 weeks. Brains were quickly removed and different regions were separated into striatum, frontal cortex, hippocampus and hypothalamus, washed with cold 0.9% saline and weighed, then immediately stored at −80°C until use. In the monoamine assay, samples were put into an appropriate volume of 0.4 mol/L perchloric acid solutions. After homogenisation and centrifugation (4°C, 15 000 × g, 10 min), supernatants were collected into tubes containing 1/2 volume of K^+ solution (20 mmol/L potassium citrate, 300 mmol/L K_2HPO_4, 2 mmol/L EDTA), then incubated at 0°C for 10 min. After centrifugation (4°C, 15 000 × g, 10 min), 20 μl of tissue homogenate supernatant was injected directly into the HPLC-ECD system.

3.8 Measurement of MAO activity

Mouse brain mitochondrial fractions were prepared following the procedure of Schurr and Livne [28]. MAO activity was assessed spectrophotometrically as described previously [29]. Briefly, the mitochondrial fraction suspended in cold sodium phosphate buffer (10 mmol/L, pH 7.4, containing 320 mmol/L sucrose), was mingled at 4°C for 20 min. The mixture was centrifuged at 15 000 × *g* for 10 min at 4°C, the supernatant was centrifuged to deposit the protein, which was suspended in the same buffer. Protein concentration was estimated by the method of Lowry *et al.* [30] using bovine serum albumin as the standard and adjusted

to 1 mg/ml. The assay mixtures contained 4 mmol/L 5-HT or 2 mmol/L β-PEA (as specific substrates for MAO-A and MAO-B, respectively), 200 μl of the mitochondrial fraction, and 10 mmol/L sodium phosphate buffer (pH 7.4) up to a final volume of 1 ml. The reaction was allowed to proceed at 37°C for 20 min, and ceased by adding 200 μl of 1 mol/L hydrochloric acid. The reaction product was extracted with 4 ml of butylacetate (for MAO-A assay) or cyclohexane (for MAO-B assay), respectively. The organic phase was measured at wavelength of 280 or 242 nm for MAO-A or MAO-B assay with spectrophotometer. Blank samples were prepared by adding 1 mol/L HCl (200 μl) prior to reaction, and were treated subsequently in the same manner.

3.9 Statistical analysis

All the values were expressed as the mean ± standard error of the mean (SEM). The effects of drugs were analysed by one-way analysis of variance (ANOVA). When significant differences ($P < 0.05$ or 0.01) were found, post hoc comparisons were made with Dunnett's test.

Downloaded At: 19:07 22 January 2011

References

[1] R. Kessler, K.A. McGonagle, S. Zhao. *Arch. Gen. Psychiatry*, **51**, 8 (1994).
[2] R. Duman, J. Malberg, S. Nakagawa. *Biol. Psychiatry*, **48**, 732 (2000).
[3] M.L. Wong, J. Licinio. *Nat. Rev. Neurosci.*, **2**, 343 (2001).
[4] W.E. Müller. *Pharmacol. Res.*, **47**, 101 (2003).
[5] I. Hindmarch. *Eur. Psychiatry*, **17**(Suppl. 3), 294 (2002).
[6] I. Malagié, D.J. David, P. Jolliet, R. Hen, M. Bourin, A.M. Gardier. *Eur. J. Pharmacol.*, **443**, 99 (2002).
[7] J.A. Gordon, R. Hen. *Annu. Rev. Neurosci.*, **27**, 193 (2004).
[8] Y.M. Wang, F. Xu, R.R. Gainetdinov, M.G. Caron. *Biol. Psychiatry*, **46**, 1124 (1999).
[9] N. Brunello, P. Blier, L.L. Judd, J. Mendlewicz, C.J. Nelson, D. Souery, J. Zohar, G. Racagni. *Int. Clin. Psychopharmacol.*, **18**, 191 (2003).
[10] H.A. D'haenen, A. Bossuyt. *Biol. Psychiatry*, **35**, 128 (1994).
[11] L. Cervo, G. Grignaschi, R. Samanin. *Eur. J. Pharmacol.*, **178**, 129 (1990).
[12] C.E. Renard, A.J. Fiocco, F. Clenet, M. Hascoet, M. Bourin. *Psychopharmacology*, **159**, 42 (2001).
[13] L. Steru, R. Chermat, B. Thierry. *Psychopharmacology*, **85**, 367 (1985).
[14] R.D. Porsolt, A. Bertin, M. Jalfre. *Arch. Int. Pharmacodyn. Ther.*, **229**, 327 (1977).
[15] Y.Q. Pei. *Epilepsia*, **24**, 177 (1983).
[16] F.P. Bymaster, W. Zhang, P.A. Carter. *Psychopharmacology*, **160**, 353 (2002).
[17] P. Blier, F.V. Abbott. *J. Psychiatry Neurosci.*, **26**, 37 (2001).
[18] E. Dailly, F. Chenu, C.E. Renard. *Fund. Clin. Pharmacol.*, **18**, 601 (2004).
[19] D.T. Wong, F.P. Bymaster. *Prog. Drug Res.*, **58**, 169 (2002).
[20] H. Saito, M. Matsumoto, H. Togashi, M. Yoshioka. *Jpn. J. Pharmacol.*, **70**, 203 (1996).
[21] J.M. Baraban, G.K. Aghajanian. *Brain Res.*, **204**, 1 (1981).
[22] Z.A. Hughes, S.C. Stanford. *Eur. J. Pharmacol.*, **317**, 83 (1996).
[23] L. Pozzi, R. Invernizzi, C. Garavaglia, R. Samanin. *J. Neurochem.*, **73**, 1051 (1999).
[24] K. Wei, W. Li, K. Koike. *J. Nat. Prod.*, **67**, 1005 (2004).
[25] L.D. Kong, C.H. Cheng, R.X. Tan. *J. Ethnopharmacol.*, **91**, 351 (2004).
[26] J. Archer. *Anim. Behav.*, **21**, 205 (1973).
[27] L.K. Zhang, X.Y. Niu, Y.W. Yu. *Acta Pharm. Sin.*, **22**, 591 (1987).
[28] A. Schurr, A. Livne. *Biochem. Pharmacol.*, **25**, 1201 (1976).
[29] Z.F. Yu, L.D. Kong, Y. Chen. *J. Ethnopharmacol.*, **83**, 161 (2002).
[30] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall. *J. Biol. Chem.*, **193**, 265 (1951).