Chinese Journal of Physiology 58(4): 219-227, 2015
DOI: 10.4077/CJP.2015.BAD283

Regulation of Intermittent Hypoxia on Brain Dopamine in Amphetaminized Rats

Po-Ling Yu[1, 2], Shu-Yun Cheng[2], Jou-Chun Chou[3], Wynn H.T. Pan[4], Shyi-Wu Wang[5, *], and Paulus S. Wang[2, 3, *]

[1]*Department of Surgery, Taipei City Hospital, Taipei 10431*
[2]*Department of Physiology, School of Medicine, National Yang-Ming University, Taipei 11221*
[3]*Medical Center of Aging Research, China Medical University Hospital, Taichung 40402*
[4]*Department of Pharmacology, School of Medicine, National Yang-Ming University, Taipei 11221*
and
[5]*Department of Physiology and Pharmacology, College of Medicine, Chang-Gung University Taoyuan 33333, Taiwan, Republic of China*

Abstract

We investigated intermittent hypoxia (IH) on dopamine (DA) release in rat brain treated with or without amphetamine (AMPH). Rats were divided into four groups including normoxia, IH, AMPH, and AMPH + IH treatments. The cerebrospinal fluid (CSF) was collected and the DA levels were detected by high performance liquid chromatography (HPLC). The plasma prolactin (PRL) concentration was measured by radioimmunoassay (RIA). We found that IH reduced basal DA concentration in media prefrontal cortex (mPFC), but increased that in striatum, where DA level was also increased in rats treated with AMPH or AMPH + IH. Angiotensin II (Ang II) increased the DA release in mPFC and striatum and this effect was enhanced in AMPH + IH group. The stimulatory effect of IH on plasma PRL was attenuated in presence of AMPH. Tyrosine hydroxylase (TH) expression was decreased by IH, but increased by AMPH + IH in mPFC. IH or AMPH treatment decreased the expression of vesicular monoamine transporter-2 (VMAT-2) in rat brain. These data suggested that IH altered the DA release and changed the protein expression levels in different parts of rat brain treated with AMPH. IH may play a role in regulating DA metabolism in AMPH addiction.

Key Words: amphetamine, angiotensin II, dopamine, intermittent hypoxia, microdialysis, prolactin

Introduction

Rats insulted by perinatal anoxia were previously shown to induce lasting changes in the function of mesocorticolimbic dopamine (DA) systems, such as a higher number of tyrosine hydroxylase (TH) immunoreactive neurons in DA cell body regions (3), increased TH activity in prefrontal cortex and nucleus accumbens (NAcc) (12) and hippocampal dentate granule cells which exacerbated methamphetamine (METH)-induced hyper-locomotion in adulthood (46). Intermittent hypoxia (IH) is one of major contributing factors to cardio-respiratory morbidities associated with sleep apneas and metabolic issues (15, 50). IH increased reactive oxygen species (ROS) ensued by the activation of TH *via* phosphorylation and increase in DA synthesis (34). Vesicular monoamine transporter (VMAT) and DA D1-receptor protein expression was upregulated within the striatum of adults rats exposed to IH insults as neonates and diminished extracellular DA observed in the post-hypoxic rat striatum which exhibited increased novelty-induced behavioral

Corresponding authors: [1] Dr. Shyi-Wu Wang, Department of Physiology and Pharmacology, College of Medicine, Chang-Gung University, Taoyuan 33333, Taiwan, R.O.C. Tel: +886-3-2118800 ext. 5253, E-mail: swwang@mail.cgu.edu.tw; and [2] Dr. Paulus S. Wang, Medical Center of Aging Research, China Medical University Hospital, Taichung 40402, Taiwan, R.O.C. Tel: +886-4-22052121 ext. 7703, E-mail: pswang3879@gmail.com
*Equal contributions to this article.
Received: March 19, 2014; Revised: August 2, 2014; Accepted: September 12, 2014.
 ISSN : 0304-4920. http://www.cps.org.tw

activation and increased basal levels of locomotor activity (11). IH prevented the nigrostriatal dopaminergic system from iron-induced oxidative injuries compared to normoxic status in rats (25).

Amphetamine (AMPH) is one of the most common illicit drug abuses in Taiwan. Among the known AMPH, METH, a potent psychostimulant known to cause neurotoxicity, is the most common psychostimulant used by abusers according to the National Bureau of Controlled Drugs, Department of Health, Taiwan, 2012. Hypoperfusion in the striatum was detected with acute and chronic METH treatment, which resulted in hypoxia and DA reduction in rats (21). However, there are limited reports on the effects of IH on DA in the presence of AMPH in the brain of rats.

Angiotensin II (Ang II) is formed in many tissues including peripheral organs and the brain. Two Ang II systems are present in the brain, *i.e.* Ang II receptors located at the circumventricular organs and cerebrovascular endothelial cells that respond to circulating Ang II, while receptors inside the blood barrier are activated by Ang II formed in the brain and/or transported to the brain from the circulation (39). Injection of Ang II into the peripheral circulation induced the effects in central nervous system, indicating the presence of receptors for this peptide in brain (9). There are two Ang II receptors, AT_1 and AT_2 receptors. The well-known biological actions of Ang II, such as contraction of smooth muscle leading to vasoconstriction and increases in blood pressure, increase in water and sodium intake, renal sodium retention and secretion of vasopressin and aldosterone are mediated by the AT_1 type (43). In the brain, both the hormonal control and the regulation of the sympathetic system are also under AT_1 receptor control (38). In rodents, but not in other mammals or humans, there are two Ang II AT_1 receptor subtypes, the AT_{1A} and AT_{1B} receptors, and these subtypes are selectively localized and regulated (18, 20). Ang II has been shown to modulate the synthesis, uptake and release of catecholamines in the sympathic neurons and the adrenal medulla as well as in central cardioregulatory areas such as the hypothalamus and the brainstem (47). Ang II receptors were found on the soma and terminals of mesolimbic dopaminergic neurons; Ang II acting through its AT_1 receptors facilitates the release of DA in the rat striatum *in vitro* and *in vivo* (8).

We have previously found that IH reduced the basal level of DA in the diencephalon and increased plasma PRL concentration (unpublished data). The purpose of this study was to investigate the effects of IH on DA levels in the striatum and media prefrontal cortex (mPFC) of rats treated with AMPH compared to rats under normoxic status. We also observed the changes of DA release by Ang II and plasma PRL which was named for one of its first known functions, the initiation and maintenance of lactation; however, this hormone is remarkably versatile, *e.g.* it regulates various events in reproduction, osmoregulation, growth, energy metabolism, immune response, brain function, behavior and angiogenesis.

Materials and Methods

Male Sprague-Dawley rats weighing 300-350 g were housed in a temperature-controlled room (22 ± 1°C) with 14 h of artificial illumination daily (0600-2000 h) and given food and water *ad libitum*. All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of National Yang-Ming University.

Animal Protocols

Rats were separated into four groups isobarically:

1. Control (Normoxia): Exposed to normoxia and intraperitoneal (IP) injection with normal saline (1 ml/kg/day) for 7 days.
2. IH: IP with normal saline (1 ml/kg/day) for 7 days then the rats were treated with 12% O_2 + 88% N_2 (1.5~2 l/min) for 8 h/day in a chamber for 7 days.
3. AMPH: IP with AMPH (5 mg/kg/day) for 7 days.
4. AMPH + IH: IP with AMPH (5 mg/kg/day) for 7 days then IH for 7 days.

Microdialysis

On the day of experiment, rats were anesthetized with chloral hydrate (loading dose 400 mg/kg, IP; supplement 80 mg/kg, IP) and were placed on a heating pad to maintain constant body temperature at 37°C throughout the experiment. Each rat was fixed on a stereotaxic apparatus (Kopf model 1430 and 1460); then the microdialysis probes were implanted. The coordinates used, from bregma, were as follows: -0.3 AP, -3.4 ML, +6.8 V from the skull for the striatum; +3.1 AP, 0.8 ML, -6.0 V from the skull for the mPFC. After insertion of three microdialysis probes into the striatum and mPFC, each was perfused with an artificial cerebrospinal fluid (aCSF: 149 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.125 mM ascorbic acid, and 5.4 mM D-glucose, pH 7.2~7.4) using a microliter syringe pump (Model 55-6206 microinjection pump, Harvard, MA, USA) at a flow rate of 1.19 μl/min. After 1 h of stabilization, dialysate samples were collected into 700-μl Eppendorf tubes at 20-min intervals, which was then injected directly into the dopamine HPLC system for DA analysis until a stable

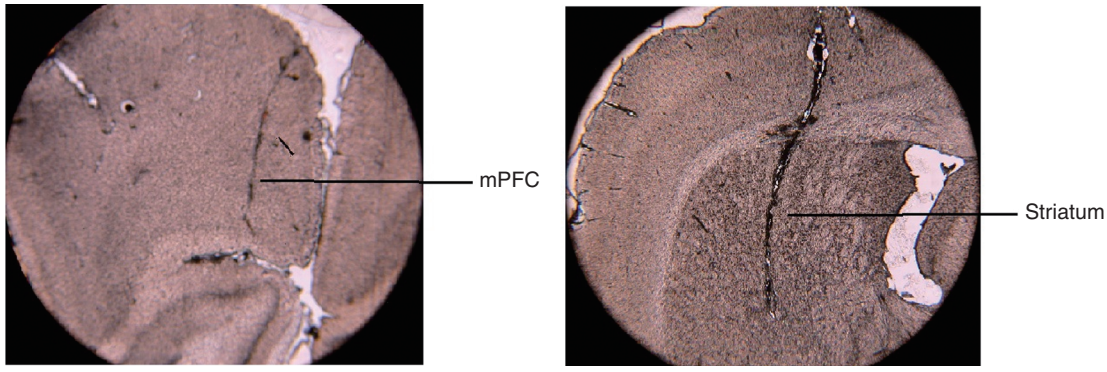

Fig. 1. Typical photomicrographs showing a microdialysis probe track implanted in the mPFC and striatum of the rat (100× magnification).

dialysate level of DA (baseline) was achieved. The changes in DA concentrations in the mPFC and striatum were monitored at 20-min intervals for 1 h. After Ang II 1 μM was injected *via* aCSF into striatum and mPFC by reverse microdialysis, the changes of DA concentrations in both areas were monitored at 20-min intervals for 2 h. HPLC-electrochemical detector for DA determination consisted of a 100 × 2.1-mm ODS Hypersil C18 5-μm column (Hewlett-Packard; no. 79916OD-552). The mobile phase (75 mM NaH_2PO_4, 1.5 mM sodium dodecyl sulfate, 0.02 mM EDTA, 100 μl/L triethylamine, 12% methanol, 13.5% acetonitrate, pH 4.5, with H_3PO_4) was delivered at 0.3 ml/min by an ISCO 260D pump. DA was detected with a glassy carbon electrode maintained at 0.75 V relative to an Ag/AgCl reference electrode. The detection limit of the DA system is 0.8 pg. The HPLC chromatogram was recorded by a linear recorder (model 1202) (10).

Histology

At the end of experiments, each rat was killed with an overdose of chloral hydrate. The brain was removed and fixed in 30% sucrose in a 10% formaldehyde/saline solution. Frozen 30-μm sections were next stained with neutral red for histological verification of the path of the probes (Fig. 1).

Radioimmunoassay (RIA) of PRL

Rats were catheterized *via* the right jugular vein 20 h before injection with saline (1 ml/kg) as a vehicle. Blood samples were collected *via* jugular vein in IH, AMPH and IH plus AMPH groups individually. An equal volume of heparinized saline was injected immediately after each bleeding. Plasma was separated by centrifugation at 8,000 × *g* for 3 min. The concentration of PRL in each plasma sample was measured by RIA as described elsewhere (34). The rat PRL kit was provided by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD, USA). Rat PRL-I-9 was used for radioiodination and rat PRL-RP-3 was used as the standard. The sensitivity of the rat PRL RIA was 3 pg per assay tube. The intra- and inter-assay coefficients of variation were 3.8% and 3.2% for the PRL RIA, respectively.

Gel Electrophoresis and Western Blotting Analysis

After decapitation, rat brain tissues mPFC, striatum, media basal hypothalamus (MBH) and cerebellum were dissected and washed twice with ice-cold 0.9% NaCl, followed by extraction for 20 min on ice with RIPA (Sigma) buffer, pH 7.4, containing 50 mM Tris, 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 5 mM EDTA (pH 8.0), 1 mM EGTA (pH 8.0), 5% β-ME and 10% protease inhibitor cocktail tablets using an ultrasonic sonicator (model XL 2020, Heat Systems, Inc, Farmingdale, NY, USA). Cell mixtures were centrifuged for 30 min at 20,000 × *g* at 4°C. The protein concentration in the supernatant was determined by the Bradford method (5). Extracted proteins were denatured by boiling for 10 min in an SDS buffer (0.125 M Tris-base, 4% SDS), 0.001% bromophenol blue, 12% sucrose and 0.15 M dithiothreitol). The proteins in the samples were separated using 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE) at 50 V for 20 min and then at 100 V for 60 min using a running buffer. The proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (NEN Life Science Products, Boston, MA, USA) using a Trans-Blot SD semi-dry-transfer cell (170-3940, Bio-Rad, Hercules, CA, USA) at 60 mA (for 8 10-mm membrane) for 40 min in a transfer solution. The membranes were washed in TBS-T buffer (0.8% NaCl, 0.02 M Trisbase, and 0.3% Tween-20, pH 7.6) for 5 min and then blocked by 120-min incubation in a blocking buffer (TBS-T buffer containing 5% nonfat dry milk). These membranes were incubated with anti-TH (1:1,000, Chemicon International, Temecula, CA, USA),

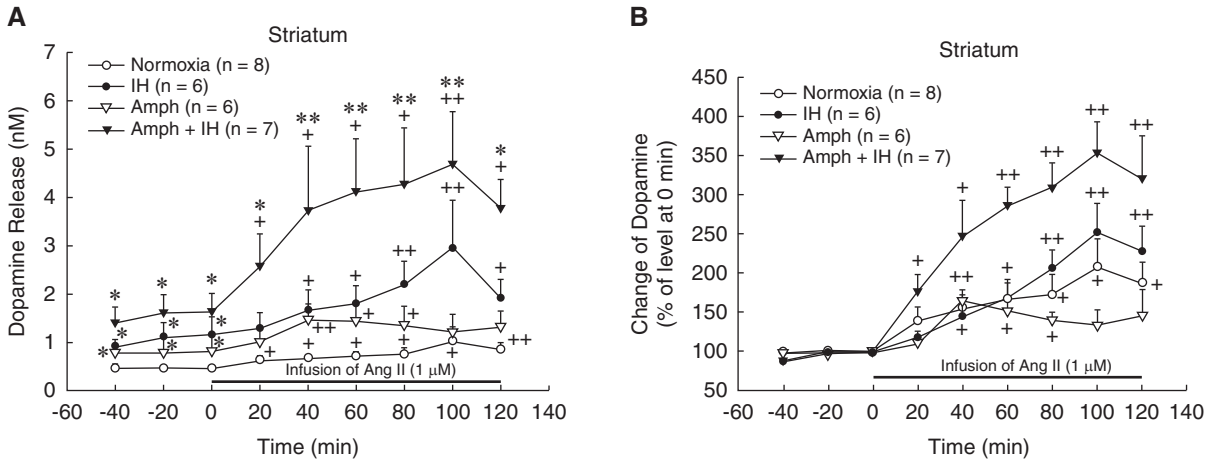

Fig. 2. Effects of infusion of Ang II (1 μM) on dopamine release in rat striatum after IH, AMPH, and AMPH + IH treatments. Each value represents mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with normoxia group. $^{+}P < 0.05$, $^{++}P < 0.01$ as compared with the group infused with vehicle.

VMAT-2 (1:1,000, Chemicon International), DAT (1:500, Chemicon International) and anti-β-actin (1:8,000) antibodies in 5% nonfat dry milk of TBS-T buffer overnight at 4°C. After three 10-min washes with TBS-T buffer, the membranes were incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG, 1:20000) in 5% nonfat dry milk of TBS-T buffer. The membranes were washed three times with TBS-T buffer, then the bands for proteins were visualized by chemiluminescence (ECL reagent Kit, Amersham, UK) (48).

Effects of Infusion of Ang II (1 μM) on the Release and Percentage Changes of DA in Rat Striatum and mPFC after IH, AMPH, AMPH + IH Treatments

Ang II (1 μM) was injected *via* aCSF into striatum and mPFC in IH, AMPH and IH plus AMPH groups individually by reverse microdialysis, the changes of DA concentrations in both areas were monitored at 20-min intervals for 2 h.

Changes of Plasma PRL Concentration in Conscious Rats Treated with IH, AMPH, AMPH + IH

Blood samples were collected *via* the jugular vein in conscious rats treated with IH, AMPH and IH plus AMPH individually. After centrifugation, the concentration of PRL in each plasma sample was measured by RIA.

Effects of Ang II (1 μM) Infusion on Plasma PRL Concentrations after IH, AMPH, AMPH + IH Treatments during Anesthetization

Ang II at 1 μM concentration was injected *via* aCSF into the brain by microdialysis probe in IH, AMPH and IH plus AMPH groups under anesthetization. Changes of plasma PRL concentrations were recorded.

Effects of IH, AMPH, AMPH + IH on the Expression of TH, VMAT-2 and DAT in mPFC, Striatum, MBH and Cerebellum by Western Blotting Analysis

After decapitation, rat brain tissues including mPFC, striatum, MBH and cerebellum were dissected and examined with TH, VMAT-2 and DAT proteins by western blotting analysis in IH, AMPH and IH plus AMPH groups respectively.

Statistic Analysis

All data were expressed as the mean ± standard error of the mean (SEM). Treatment means were tested for homogeneity using the analysis of variance (ANOVA), and the differences between the specific means were tested for the significance by Duncan's multiple range test. The level of significance chosen was $P < 0.05$.

Results

Effects of Infusion of Ang II (1 μM) on the Release and Percentage Change of DA in Rat Striatum after IH, AMPH, AMPH + IH Treatments

The basal DA release was increased in IH, AMPH and AMPH + IH groups compared to the normoxia status ($^{*}P < 0.05$, Fig. 2A). The DA release increased in all four groups treated with Ang II (1 μM) for 20, 40, 60, 80 and 100 min compared to the vehicle ($^{+}P < 0.05$, $^{++}P < 0.01$, Fig. 2, A and B); DA released in-

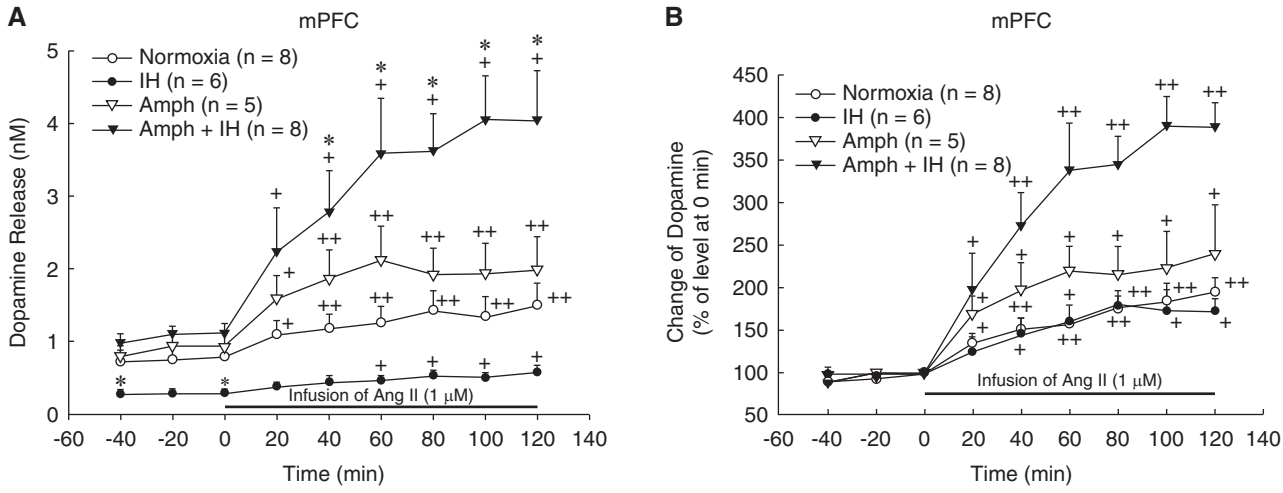

Fig. 3. Effects of infusion of Ang II (1 μM) on dopamine release in rat mPFC after IH, AMPH, and AMPH + IH treatments. Each value represents mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with normoxia group. $^{+}P < 0.05$, $^{++}P < 0.01$ as compared with the group infused with vehicle.

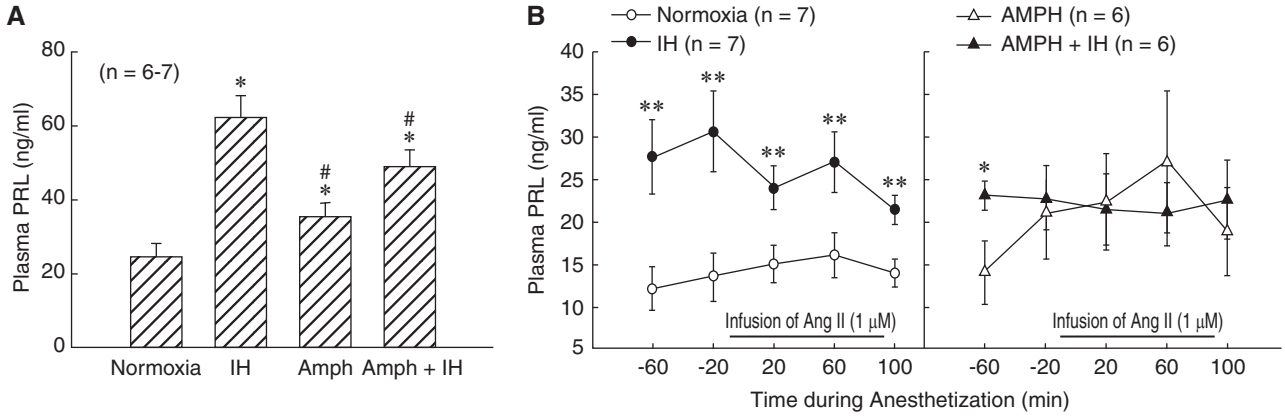

Fig. 4. Concentration of plasma PRL in conscious rats treated with IH, AMPH, and AMPH + IH and effects of Ang II (1 μM) infusion *via* microdialysis probe on plasma PRL concentrations during anesthetization. Each value represents mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with normoxia group, or AMPH group, $^{\#}P < 0.05$ as compared with IH group.

creased significantly in AMPH + IH group compared to the normoxia status ($^{**}P < 0.01$, Fig. 2A).

Effects of Infusion of Ang II (1 μM) on the Release and Percentage Change of DA in Rat mPFC after IH, AMPH, AMPH + IH Treatments

The basal DA release was decreased in IH group compared to the normoxia status ($^{*}P < 0.05$, Fig. 3A. The DA release increased in all four groups treated with Ang II (1 μM) for 20, 40, 60, 80 and 100 min compared to the vehicle ($^{+}P < 0.05$, $^{++}P < 0.01$, Fig. 3, A and B); the DA released increased significantly in AMPH + IH group compared to the normoxia status ($^{**}P < 0.01$, Fig. 3A).

Plasma Concentration of PRL in Conscious Rats Treated with IH, AMPH, AMPH + IH and the Effects of Ang II (1 μM) Infusion on Plasma PRL Concentrations

The plasma PRL concentration increased in IH, AMPH and AMPH + IH groups compared to the normoxia status in conscious rats ($^{*}P < 0.05$, Fig. 4A). However, the stimulatory effect of IH on plasma PRL was attenuated in the presence of AMPH ($^{\#}P < 0.05$, Fig. 4A). The plasma PRL concentrations decreased gradually in the four groups in which rats were anesthetized in spite of the presence of Ang II (Fig. 4B).

Effects of IH, AMPH, AMPH + IH on the Expression of TH, VMAT-2 and DAT in mPFC, Striatum, MBH and Cerebellum by Western Blotting Analysis

In the mPFC and MBH areas, the expression of TH increased in the presence of AMPH compared to the normoxia; the reaction sustained in the presence

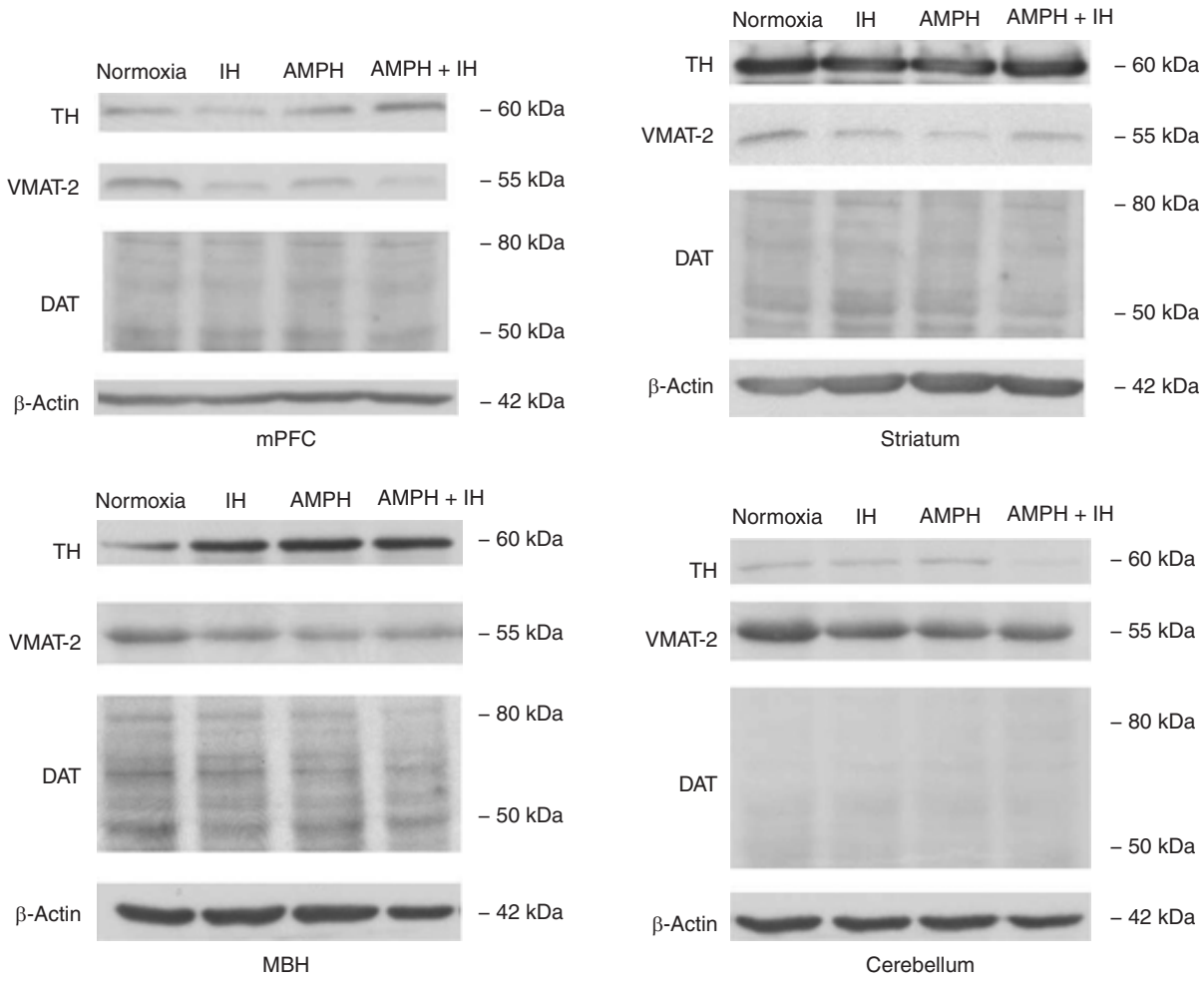

Fig. 5. Effects of IH, AMPH, and AMPH + IH on the expression of TH, VMAT-2, and DAT in mPFC, striatum, MBH and cerebellum.

of AMPH + IH. The expression of VMAT-2 decreased in the presence of IH, AMPH or AMPH + IH compared to the normoxia in all four areas of rats brain. No change of DAT expression was noted in different situations in rats brain (Fig. 5).

Discussion

AMPH is believed to increase DA release from the striatum by different mechanisms including increasing cytosolic DA by redistribution of neurotransmitters *via* VMAT-2 from synaptic vesicles to the neuronal cytoplasm and the reverse transport of DA from the cytoplasm into the synapse *via* the DAT (45), competitively binding with DAT by facilitating exchange of cytosolic DA or burst channel like transporter to DA efflux (19) and inhibiting the monoamine oxidase to decrease the metabolism of postsynaptic DA (13). In rodents, long-term low-dose administration of AMPH induced the release of DA from the striatum while high doses of AMPH decreased DA release from the striatum (35). Intraperitoneal administration with varying low doses of methylphenidate, an analog of AMPH, at 0.25, 0.5 and 1.0 mg/kg, produced dose-dependent increases in extracellular DA levels within NAcc (2). However, repeated high-dose administrations of the METH caused persistent dopaminergic deficits, *i.e.*, reductions in striatal DA content, DAT density and/or activity of the DA synthesizing enzyme, TH, in rodents (17).

The striatum is one of the brain areas most sensitive to deficiency in oxygen even when the brain energy metabolism is unaffected by a moderate hypoxia (41). Rodents subjected to chronic IH showed sprouting of noradrenergic terminals in multiple motor and sensory regions of the lower brainstem which were immunohistochemically-stained with dopamine-β-hydroxylase, a marker for norepinephrine (27). Rats exposed to various degrees of hypoxia (15, 10 and 8% O_2 in N_2) increased DA levels in striatal dialysates to 200, 400 and 1,100%, respectively. This reaction was inhibited by nomifensine, a blocker of dopamine reuptake (1). DAT was involved in the onset of hypoxia-induced DA efflux in the striatum by *in vivo* microdi-

alysis (31). Enhanced NAcc DA function was noted in adult rats subjected to intrauterine anoxia during cesarean section birth; on the other hand, DA expression in mPFC was decreased possibly by the inhibitory effects of NAcc (6, 7). Similarly, our unpublished data showed that IH decreased the DA release in diencephalon compared to the normoxia status opposite to the effects on the striatum in this study. Moreover, IH plus AMPH enhanced the DA release more significantly than AMPH or IH alone in both the striatum and diencephalon (Figs. 2 and 3). Hopefully, IH might be clinically used in treating the deficiency of DA in AMPH addiction.

Patients with obstructive sleep apnea (OSA) are exposed to chronic intermittent hypoxia (CIH), which is thought to be the underlying mechanism that links OSA with an increased risk of cardiovascular disease such as increasing arterial blood pressure. Following exposure to CIH, rats have increased noradrenergic terminal density in brain stem sensory and motor nuclei and upregulated expression of the excitatory α1-adrenergic receptors in the hypoglossal motor, trigeminal sensory and motor nuclei (27). This suggests that CIH may enhance central catecholaminergic transmission. On the other hand, sympathetic nerve activity, the plasma concentration of Ang II and the vasoconstrictor response to Ang II are elevated in OSA patients (22, 28). Arterial pressure increased significantly after exposure to IH in healthy human subjects; however, the increase in blood pressure was abolished by blockade of the AT_1 receptor (14). Local administration of Ang II in the rat striatum enhanced the release of DA (42); moreover, local infusion of Ang II amplified the effects of IH, AMPH or both on release of DA in striatum and mPFC in rats in our study. DA depletion induced upregulation of AT_1 in basal ganglion of rodents; however, candesartan (an AT_1 blocker) attenuated AMPH-induced behavioral sensitization in rats by affecting DA neurotransmission in the NAcc and the caudate-putamen (32, 44). Direct AT_2 receptor stimulation decreases DA synthesis in the rat striatum. Rats daily treated with candesartan protected dopaminergic neurons of the nigrostriatal tract against the neurotoxin-induced cell death. Stimulation of the AT_1 receptor with Ang II increased the formation of hydroxyl radicals in striatum (26). Chronic treatment with losartan, a AT_1 receptor blocker, in dystrophic hamsters stimulated frequency of breathing and increased dopamine D1 receptor density in the striatum indicating a functional interaction between AT_1 receptors and D1 receptors that may modulate ventilation (40).

The plasma PRL increased at 24 h and became significant when rats were treated in hypoxic status (49). In human, plasma PRL decreased in men under prolonged exposure (3-4 days) to an altitude of 4,350 m compared to normoxia (37); however, PRL level increased in male elite climbers after high-altitude chronic hypoxia exposure (7 weeks at 5,200 m) in comparison with climbers at sea level (4). Elevated plasma PRL was noted in methamphetamine-dependent human in difference with healthy control (51). CART peptide, a cocaine- and amphetamine-regulated transcript identified as the mRNA that was elevated in rat striatum following acute administration of cocaine or AMPH, suppressed PRL released from dispersed rats anterior pituitary cells in a dose-related manner (23). Centrally-administered Ang II appears to be inhibitory to PRL secretion in male as well as female rats, regardless of the endocrine status of the animal (29, 30). The plasma PRL decreased following microinjections of Ang II in the medial preoptic area of rats (24). Similarly, the plasma PRL concentrations decreased gradually in the four groups of rats which were in an anesthetized status in spite of the presence of Ang II (Fig. 4B).

TH, a hypoxia-regulated gene, was found to be involved in tissue adaptation to hypoxia. IH increased DA levels in the rat brain stem due in part to increasing synthesis *via* activation of the rate-limiting synthesizing enzymes, TH (34). TH activity increased in the rat cortex in sustained hypoxia than IH or normoxia while vice versa was true in brain stem. Such difference indicated that this enzyme activity changed in differential regional brain susceptibility to different hypoxic exposures (16). Rats exposed to IH (15 s) (15 s in 5% O_2 then 5 min in 21% O_2) caused a robust increase in TH activity, DA level in the medulla then IH (90 s) (90 s each in 10% O_2 & 21% O_2). Also, IH (15 s) increased the generation of ROS in brain stem which was nearly threefold higher than that evoked by IH (90 s) (34). In Western blotting, the present study showed that the expression of TH decreased in mPFC but increased in MBH when rats were treated with IH; however, TH increased in the presence of AMPH plus IH in mPFC and MBH areas. Expression of VMAT-2 decreased in the presence of IH, AMPH or both compared to the normoxia in the brain of rats. These might explain the difference of DA release in mPFC and the striatum in this study. Striatal expression of VMAT-2 and D1 receptor proteins was increased in consequence of persistent reductions in extracellular levels of DA in post-hypoxic rat pups (11). Similarly, decreased expression of TH, VMAT-2 and DAT was noted in rat striatum treated with AMPH, which caused the depletion of DA (48). Hypoxia and reduction in TH expression caused by hypoperfusion were detected in rat striatum treated with chronic METH (21).

In summary, our study suggested that IH altered the DA release and changed the protein expression patterns in different parts of rat brain treated with

AMPH and this phenomenon was amplified in the presence of Ang II. Interestingly, IH plus AMPH enhanced the DA release more significantly than AMPH or IH alone in both striatum and diencephalon. IH and Ang II may be shown to play a role in regulating DA metabolism in AMPH addiction in future.

Acknowledgments

This work was supported by the grant No. MOST 103-2320-B-039-034-MY2 from the Ministry of Science and Technology, Taiwan, ROC.

References

1. Akiyama, Y., Koshimura, K., Ohue, T., Lee, K., Miwa, S., Yamagata, S. and Kikuchi, H. Effects of hypoxia on the activity of the dopaminergic neuron system in the rat striatum as studied by *in vivo* brain microdialysis. *J. Neurochem.* 57: 997-1002, 1991.
2. Benso, A., Broglio, F., Aimaretti, G., Lucatello, B., Lanfranco, F., Ghigo, E. and Grottoli, S. Endocrine and metabolic responses to extreme altitude and physical exercise in climbers. *Eur. J. Endocrinol.* 157: 733-740, 2007.
3. Berridge, C.W., Devilbiss, D.M., Andrzejewski, M.E., Arnsten, A.F., Kelley, A.E., Schmeichel, B., Hamilton, C. and Spencer, R.C. Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biol. Psychiat.* 60: 1111-1120, 2006.
4. Bjelke, B., Andersson, K., Ogren, S.O. and Bolme, P. Asphyctic lesion: proliferation of tyrosine hydroxylase-immunoreactive nerve cell bodies in the rat substantia nigra and functional changes in dopamine neurotransmission. *Brain Res*. 543: 1-9, 1991.
5. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254, 1976.
6. Brake, W.G., Flores, G., Francis, D., Meaney, M.J., Srivastava, L.K. and Gratton, A. Enhanced nucleus accumbens dopamine and plasma corticosterone stress responses in adult rats with neonatal excitotoxic lesions to the medial prefrontal cortex. *Neuroscience* 96: 687-695, 2000.
7. Brake, W.G., Sullivan, R.M. and Gratton, A. Perinatal distress leads to lateralized medial prefrontal cortical dopamine hypofunction in adult rats. *J. Neurosci.* 20: 5538-5543, 2000.
8. Brown, D.C., Steward, L.J., Ge, J. and Barnes, N.M. Ability of angiotensin II to modulate striatal dopamine release *via* the AT_1 receptor *in vitro* and *in vivo*. *Brit. J. Pharmacol.* 118: 414-420, 1996.
9. Buckley, J.P. The central effects of the renin-angiotensin system. *Clin. Exp. Hypertens*. 10: 1-16, 1988.
10. Chen, N.N. and Pan, W.H. Regulatory effects of D2 receptors in the ventral tegmental area on the mesocorticolimbic dopaminergic pathway. *J. Neurochem*. 74: 2576-2582, 2000.
11. Chia, M., Liao, C.A., Huang, C.Y., Lee, W.C., Hou, C.W., Yu, S.H., Harris, M.B., Hsu, T.S., Lee, S.D. and Kuo, C.H. Reducing body fat with altitude hypoxia training in swimmers: role of blood perfusion to skeletal muscles. *Chinese J. Physiol*. 56: 18-25, 2013.
12. El-Khodor, B.F. and Boksa, P. Effects of birth insult and stress at adulthood on *in vivo* tyrosine hydroxylase and tryptophan hydroxylase activities in rat brain. *Synapse* 47: 87-89, 2003.
13. Flatmark, T. Catecholamine biosynthesis and physiological regulation in neuroendocrine cells. *Acta Physiol. Scand.* 168: 1-17, 2000.
14. Foster, G.E., Hanly, P.J., Ahmed, S.B., Beaudin, A.E., Pialoux, V. and Poulin, M.J. Intermittent hypoxia increases arterial blood pressure in humans through a Renin-Angiotensin system-dependent mechanism. *Hypertension* 56: 369-377, 2010.
15. Foster, G.E., Poulin, M.J. and Hanly, P.J. Intermittent hypoxia and vascular function: implications for obstructive sleep apnoea. *Exp. Physiol*. 92: 51-65, 2007.
16. Gozal, E., Shah, Z.A., Pequignot, J.M., Pequignot, J., Sachleben, L.R., Czyzyk-Krzeska, M.F., Li, R.C., Guo, S.Z. and Gozal, D. Tyrosine hydroxylase expression and activity in the rat brain: differential regulation after long-term intermittent or sustained hypoxia. *J. Appl. Physiol.* 99: 642-649, 2005.
17. Hotchkiss, A.J., Morgan, M.E. and Gibb, J.W. The long-term effects of multiple doses of methamphetamine on neostriatal tryptophan hydroxlase, tyrosine hydroxylase, choline acetyltransferase and glutamate decarboxylase activities. *Life Sci.* 25: 1373-1378, 1979.
18. Inagami, T., Guo, D.F. and Kitami, Y. Molecular biology of angiotensin II receptors: an overview. *J. Hypertens.* 12: 583-594, 1994.
19. Kahlig, K.M., Binda, F., Khoshbouei, H., Blakely, R.D., McMahon, D.G., Javitch, J.A. and Galli, A. Amphetamine induces dopamine efflux through a dopamine transporter channel. *Proc. Natl. Acad. Sci. USA* 102: 3495-3500, 2005.
20. Kakar, S.S., Sellers, J.C., Devor, D.C., Musgrove, L.C. and Neill, J.D. Angiotensin II type-1receptor subtype cDNAs: differential tissue expression and hormonal regulation. *Biochem. Biophys. Res. Commun.* 183: 1090-1096, 1992.
21. Kousik, S.M., Graves, S.M., Napier, T.C., Zhao, C. and Carvey, P.M. Methamphetamine-induced vascular changes lead to striatal hypoxia and dopamine reduction. *Neuroreport* 22: 923-928, 2011.
22. Kraiczi, H., Hedner, J., Peker, Y. and Carlson, J. Increased vasoconstrictor sensitivity in obstructive sleep apnea. *J. Appl. Physiol.* 89: 493-498, 2000.
23. Kuriyama, G., Takekoshi, S., Tojo, K., Nakai, Y., Kuhar, M.J. and Osamura, R.Y. Cocaine- and amphetamine-regulated transcript peptide in the rat anterior pituitary gland is localized in gonadotrophs and suppresses prolactin secretion. *Endocrinology* 145: 2542-2550, 2004.
24. Leite, C.M., Machado, G.J., Dornelles, R.C. and Franci, C.R. Actions of angiotensin II and dopamine in the medial preoptic area on prolactin secretion. *Physiol. Res.* 57: 109-118, 2008.
25. Liao, C.H., Chen, S.Y., Kuo, J.S. and Pang, C.Y. Reduction of motor disorder in 6-OHDA-induced severe parkinsonism rats by post treatment with granulocyte-colony stimulating factor. *Chinese J. Physiol*. 56: 147-154, 2013.
26. Mertens, B., Vanderheyden, P., Michotte, Y. and Sarre, S. Direct angiotensin II type 2 receptor stimulation decreases dopamine synthesis in the rat striatum. *Neuropharmacology* 58: 1038-1044, 2010.
27. Mody, P., Rukhadze, I. and Kubin, L. Rats subjected to chronic-intermittent hypoxia have increased density of noradrenergic terminals in the trigeminal sensory and motor nuclei. *Neurosci. Lett.* 505: 176-179, 2011.
28. Moller, D.S., Lind, P., Strunge, B. and Pedersen, E.B. Abnormal vasoactive hormones and 24-hour blood pressure in obstructive sleep apnea. *Am. J. Hypertens.* 16: 274-280, 2003.
29. Myers, L.S. and Steele, M.K. The brain renin-angiotensin system and prolactin secretion in the male rat. *Endocrinology* 129: 1744-1748, 1991.
30. Myers, L.S. and Steele, M.K. The brain renin-angiotensin system and the regulation of prolactin secretion in female rats: influence of ovarian hormones. *J. Neuroendocrinol.* 1: 299-303, 1989.
31. Orset, C., Parrot, S., Sauvinet, V., Cottet-Emard, J.M., Bérod, A., Pequignot, J.M. and Denoroy, L. Dopamine transporters are involved in the onset of hypoxia-induced dopamine efflux in striatum as revealed by *in vivo* microdialysis. *Neurochem. Int.* 46: 623-633, 2005.

32. Paz, M.C., Assis, M.A., Cabrera, R.J., Cancela, L.M. and Bregonzio, C. The AT_1 angiotensin II receptor blockade attenuates the development of amphetamine-induced behavioral sensitization in a two-injection protocol. *Synapse* 65: 505-512, 2011.
33. Pu, H.F., Yang, M.Y., Wang, S.W., Kao, Y.C., Jeng, F.S. and Wang, P.S. Effects of thyroidectomy on the metablic clearance rate of prolactin and prolactin release in the rats. *Chinese J. Physiol.* 30: 15-24, 1987.
34. Raghuraman, G., Rai, V., Peng, Y.J., Prabhakar, N.R. and Kumar, G.K. Pattern-specific sustained activation of tyrosine hydroxylase by intermittent hypoxia: role of reactive oxygen species-dependent downregulation of protein phosphatase 2A and upregulation of protein kinases. *Antioxid. Redox Signal.* 11: 1777-1789, 2009.
35. Ricaurte, G.A., Seiden, L.S. and Schuster, C.R. Further evidence that amphetamines produce long-lasting dopamine neurochemical deficits by destroying dopamine nerve fibers. *Brain Res.* 303: 359-364, 1984.
36. Richalet, J.P., Letournel, M. and Souberbielle, J.C. Effects of high-altitude hypoxia on the hormonal response to hypothalamic factors. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 299: R1685-R1692, 2010.
37. Rukhadze, I., Fenik, V.B., Benincasa, K.E., Price, A. and Kubin, L. Chronic intermittent hypoxia alters density of aminergic terminals and receptors in the hypoglossal motor nucleus. *Am. J. Respir. Crit. Care Med*. 15: 1321-1329, 2010.
38. Saavedra, J.M. Brain and pituitary angiotensin. *Endocr. Rev.* 13: 329-380, 1992.
39. Saavedra, J.M. Brain angiotensin II: new developments, unanswered questions and therapeutic opportunities. *Cell. Mol. Neurobiol*. 25: 485-512, 2005.
40. Schlenker, E.H. In dystrophic hamsters losartan affects control of ventilation and dopamine D1 receptor density. *Respir. Physiol. Neurobiol.* 173: 71-78, 2010.
41. Smith, M.L., Auer, R.N. and Siesjo, B.K. The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. *Acta Neuropathol*. 64: 319-332, 1984.
42. Stragier, B., Sarre, S., Vanderheyden, P., Vauquelin, G., Fournie-Zaluski, M.C., Ebinger, G. and Michotte, Y. Metabolism of angiotensin II is required for its *in vivo* effect on dopamine release in the striatum of the rat. *J. Neurochem.* 90: 1251-1257, 2004.
43. Timmermans, P.B., Wong, P.C., Chiu, A.T., Herblin, W.F., Benfield, P., Carini, D.J., Lee, R.J., Wexler, R.R., Saye, J.A. and Smith, R.D. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol. Rev.* 45: 205-251, 1993.
44. Villar-Cheda, B., Rodríguez-Pallares, J., Valenzuela, R., Muñoz, A., Guerra, M.J., Baltatu, O.C. and Labandeira-Garcia, J.L. Nigral and striatal regulation of angiotensin receptor expression by dopamine and angiotensin in rodents: implications for progression of Parkinson's disease. *Eur. J. Neurosci.* 32: 1695-1706, 2010.
45. Wagner, G.C., Ricaurte, G.A., Seiden, L.S., Schuster, C.R., Miller, R.J. and Westley, J. Long-lasting depletions of striatal dopamine and loss of dopamine uptake sites following repeated administration of methamphetamine. *Brain Res.* 181: 151-160, 1980.
46. Wakuda, T., Matsuzaki, H., Suzuki, K., Iwata, Y., Shinmura, C., Suda, S., Iwata, K., Yamamoto, S., Sugihara, G., Tsuchiya, K.J., Ueki, T., Nakamura, K., Nakahara, D., Takei, N. and Mori, N. Perinatal asphyxia reduces dentate granule cells and exacerbates methamphetamine-induced hyperlocomotion in adulthood. *PLoS One* 3: e3648, 2008.
47. Yu, K., Lu, D., Rowland, N.E. and Raizada, M.K. Angiotensin II regulation of tyrosine hydroxylase gene expression in the neuronal cultures of normotensive and spontaneously hypertensive rats. *Endocrinology* 137: 3566-3576, 1996.
48. Yu, P.L., Wu, C.I., Lee, T.S., Pan, W.H., Wang, P.S. and Wang, S.W. Attenuation of estradiol on the reduction of striatal dopamine by amphetamine in ovariectomized rats. *J. Cell. Biochem.* 108: 1318-1324, 2009.
49. Zhang, Y.S. and Du, J.Z. The response of growth hormone and prolactin of rats to hypoxia. *Neurosci. Lett.* 279: 137-140, 2000.
50. Zhou, J.J., Wang, S. and Zhang, Y. Benefit or damage of intermittent hypoxia on insulin resistance. *Adapt. Med.* 5: 114-124, 2013.
51. Zorick, T., Mandelkern, M.A., Lee, B., Wong, M.L., Miotto, K., Shabazian, J. and London, E.D. Elevated plasma prolactin in abstinent methamphetamine-dependent subjects. *Am. J. Drug Alcohol Abuse* 37: 62-67, 2011.