Effect of Bak Foong Pills on Enhancing Dopamine Release from the Amygdala of Female Rats

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To investigate whether Bak Foong Pills (BFP), a well-known gynaecological tonic, has a direct effect on the central nervous system, we employed the *in vivo* electrochemical detection technique, fast cyclic voltammetry (FCV), to measure the dopamine release from the mesolimbic structure-amygdala of both male and female rats. The results showed that intracerebroventricular BFP (0.75, 1.5 μg) treatment promoted dopamine release from the amygdala in both female and ovariectomized female rats. The BFP-induced response appeared within 5 min after addition of BFP and lasted for at least 40 min. However, no effect of BFP was observed in male rats for an observed period of up to 60 min. The results suggest that BFP may have gender-specific beneficial effect on dopaminergic functions of the amygdala.

Key words Bak Foong Pill; dopamine; amygdala; rat

Gonadal steroid hormones, especially estrogen, have been demonstrated to exert substantial modulatory effects upon dopaminergic system.[1)] Research has shown that the maximum release of dopamine (DA) from the amygdala is gender specific, with higher release in the female than the male rats.[2)] It is thought that estrogen plays an important role in modulating dopamine functions, since estrogen is well known to have neuroprotective effects on the central nervous system,[3,4)] including an effect on the metabolism of DA. However, there are limitations in the neuroprotection offered by hormone therapy because of its potential side effects. Here we examined the effects of a traditional Chinese medicine with reputed estrogen-like properties to see if an alternative therapy could be found.

Bak Foong Pills (BFP, also known as Bai Feng Wan) is an over-the-counter traditional Chinese medicine (China registration #Z980035) used for treatment of various gynaecological disorders, including dysmenorrhoea, irregular menstruation and general weakness after childbirth. It has long been suggested that BFP is an estrogen-like medicine. Indeed, many of the properties of BFP are similar to those of estrogen, such as its ability to reduce blood pressure, increase vasorelaxation, reduce serum triglyceride,[5)] increase cystic fibrosis transmembrane-conductance regulator (CFTR) expression,[6)] and its anti-platelet activity.[7)] There have also been some observations that BFP action may be dependent on the presence of the ovaries in female animals and its action may therefore be *via* the hypothalamus-pituitary-ovary axis (personal communication). Apart from its well-known effect on the female reproductive tract, BFP has also been shown to exert a direct stimulatory effect on gastrointestinal Cl^- secretion by predominantly activating adenylate cyclase and apical cAMP-dependent Cl^- channels.[8)] BFP is also known to have beneficial effects on overall body function including clinically observed beneficial effects on the neuropatic syrigmus and stroke sequela-dementia.[9)] It seems likely that BFP may have a direct effect on the central nervous system.

In order to have better understanding of the action of BFP on the central nervous system, we employed the fast cyclic voltammetry (FCV) to examine the direct effect of BFP on DA release from the amygdala of rats. Our study also aimed to determine whether there is a gender difference in the action of BFP.

MATERIALS AND METHODS

Animals Adult male and female Wistar rats (200—250 g bodyweight) were used in the experiment. Half of the female rats were ovariectomized bilaterally under chloral hydrate (400 mg/kg i.p.) anesthesia and left to recover for 2 weeks. Vaginal smear was done every day for 6 d to ensure the procedure was successful. Animals were maintained on a 12 h light–dark cycle with food and water being freely available. All the male, female or ovariectomized female rats were divided into four groups (6 in each group), including BFP treatment (0.75, 1.5 μg), obtained from Eu Yan Sang (HK) Ltd., vehicle (sterile saline) treatment or without treatment group. During the experiments, 2.5 μl of BFP or vehicle was injected intracerebroventricularly within 5 min.

Electrical Stimulation of Ventral Tegmental Area (VTA) Anaesthetized with chloral hydrate, rats were fixed in a stereotaxic apparatus. Holes were drilled in the skull for reference, stimulating and working electrodes. The stereotaxic coordinates are given in millimeters according to the atlas of Paxinos and Watson. The standard concentric bipolar stimulating electrode was positioned in the VTA (AP: -5.8 mm posterior to bregma; L: $+0.5$ mm lateral to the sagittal sinus; V: -6.5 mm below the surface of the cortex). The working electrode was placed in the amygdala at AP: -2.5 mm; L: $+3.6$ mm; V: -7.8 mm. A miniature silver–silver chloride (Ag/AgCl) electrode served as the reference. This was placed in a small dip in the skull contralateral to the working electrode. The stimulating electrode was lowered with continuous stimulation of 200 pulses, 1.5 mA intensity, 0.2 ms pulse width at 100 Hz until DA release was detected in the amygdala. Each of the stimulation was applied to the VTA at intervals of 5 min throughout the experiment, and recorded for 70 min. BFP or saline was injected after the stable electric-stimulated release of DA.

Fast Cyclic Voltammetry (FCV) Single carbon fibre

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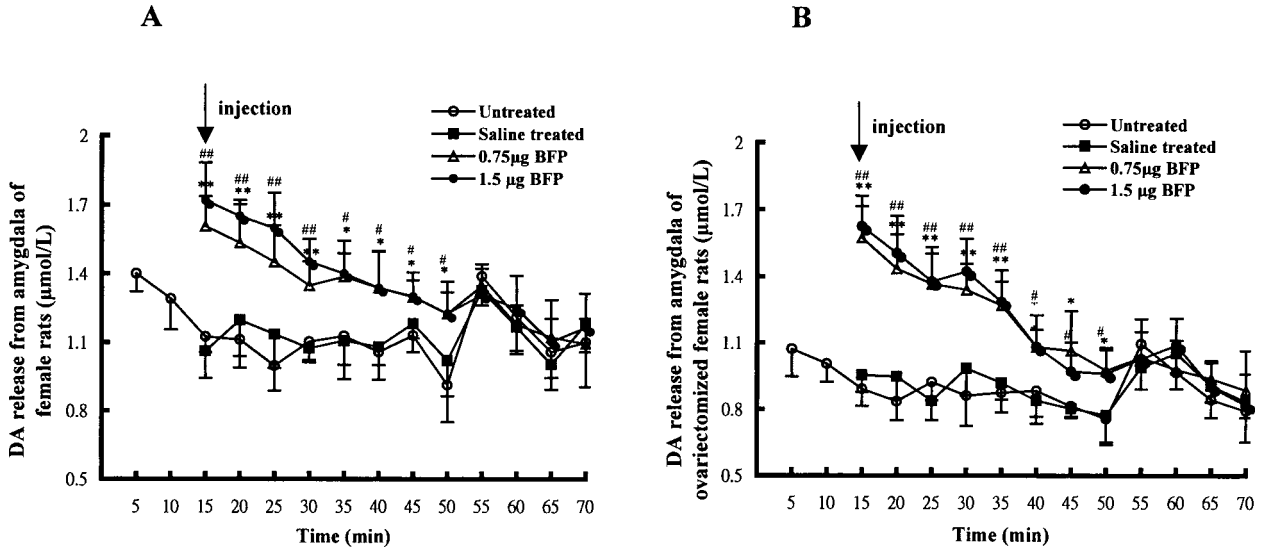

Fig. 1. Effect of Intracerebroventricular BFP Treatment (0.75, 1.5 μg) on Dopamine Release from the Amygdala of Female (A) and Ovariectomized Female Rats (B), with Untreated and Saline-Treated Controls

n=6. $^{*}p<0.05$, $^{**}p<0.01$, 0.75 μg BFP treatment group *versus* saline treated group; $^{\#}p<0.05$, $^{\#\#}p<0.01$, 1.5 μg BFP treatment group *versus* saline treated group.

microelectrodes (8 μm in diameter) were prepared. The carbon fiber protruding beyond the glass insulation was cut to a length of 20—60 μm under micromanipulator control. All electrochemical measurements were made using FCV which was performed using a Millar Voltammetric Analyser (PD Systems, West Molesey, Surrey). The input voltage to the working electrode consisted of 1.5 cycles triangular waveform (−1.0—+1.4 V *vs.* Ag/AgCl) sweeping initially in the negative direction. With these electrochemical parameters, DA giving an oxidation peak at +600 mV was monitored with sample-and-hold circuits for each working electrode.[2] The outputs from the sample-and-hold circuits were displayed on a y–t chart recorder of computer and an oscilloscope screen through a specific CED 1401 interface. Data were stored as files using CED software (Signal Averager and Chart, PD Systems, West Molesey, Surrey).

At the end of each experiment, the working electrodes were calibrated in solutions of DA with different concentrations in phosphate-buffered saline. The rat brains were also removed and checked for correct location of the injection needles.

Statistical Analysis Data are expressed as mean±standard error of the mean (S.E.M.) where applicable, and n is the number of rats. Significance testing used was Student's t test and one-way analysis of variance (ANOVA). The significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

It has been shown that the amygdala generates important projections controlling endocrine and autonomic functions, regulating different aspects of integrated emotional responses, long-term memory and sexual differentiation. The amygdala is full of activities of neurotransmitters, especially dopamine. The dopamine-containing neurons innervating the amygdala originate primarily from the substantia nigra and VTA.[10] DA release can be detected from the amygdala by electrical stimulation of VTA using FCV. The present study

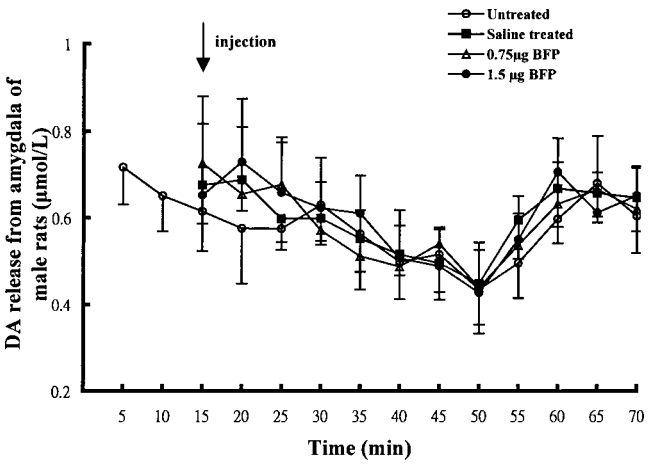

Fig. 2. Effect of Intracerebroventricular BFP Treatment (0.75, 1.5 μg) on Dopamine Release from the Amygdala of Male Rats, with Untreated and Saline-Treated Controls

n=6.

has demonstrated that BFP exerts an effect on amygdaloid DA release. DA release from the amygdala of female rats was enhanced by intracerebroventricular BFP treatment at dose of 0.75 and 1.5 μg (Fig. 1A). Treatment with the same volume of saline did not affect DA release, which was similar to that observed in the non-treated control group. Interestingly, the results obtained from male and female rats are different. The effect of BFP on DA release was not observed in the male rats (Fig 2). In addition, the basal release of DA is also different, with more DA release observed in the female rats, consistent with our previously observed difference in DA release between two sexes.[2] Noteworthy is that 17-β estradiol potentiate the striatal DA release of female rats but not that of male rats.[11] Gender differences also appeared in the modulatory effects of estrogen in castrated male and female rats.[12] These observations suggest that the effects of BFP and estrogen are similar. The gender-specific effect of

BFP on DA release in rats could be due to sexual dimorphic differentiation of the brain.[13,14]

As a traditional Chinese medicine, BFP has long been suggested to be an estrogen-like medicine by modulating the hypothalamus-pituitary-ovary axis. In order to explore whether or not endogenous estrogen has a role in the dopamine release after BFP treatment, we examined the effect of BFP on ovariectomized rats. The results showed that the basal release of DA was lower in ovariectomized rats (Fig. 1B) when compared to that in normal female rats (Fig. 1A), which is consistent with previously observed effect of estrogen on DA release.[3] However, our study also showed that BFP-induced amygdaloid DA release in the ovariectomized rats is similar to that in non-ovariectomized rats, suggesting that the action of BFP is not ovary-dependent. In other words, the gender-specific effect of BFP appears to be modulated by extra-ovary mechanism(s). It should be noted that the source of estrogen in the brain is thought to be produced in extra-gonadal sites,[15] which may contribute to the gender-specific effect of BFP observed. On the other hand, research has shown that there are no differences in brain estrogen receptor-beta expression two weeks following ovariectomy.[16] This suggests that gender difference in estrogen receptor expression[17] may still be retained in our ovariectomized rats, accounting for the gender-specific effect of BFP. While the mechanism mediating the effect of BFP is not clear at the moment, it appears that BFP exerts an effect on the dopominergic tissue of the amygdala, most likely acting on specific receptors, since DA release from the amygdala in normal and ovariectomized rats was increased significantly 5 min after BFP injection, which then returned to the basal level after 40 min, and a second BFP challenge within the experimental period (data not shown) could not elicit any further response, which could be due to receptor desensitisation.

It has been demonstrated that estrogen has neuroprotective effects on the central nervous system.[18] However, estrogen replacement therapy is not a viable option for all the patients because of potential side effects. Recently, various phytoestrogens have been developed to retain their favourable actions but minimize the adverse side effects of estrogens.[19] As a herbal mixture, BFP may contain phytoestrogens, the details of which remains to be elucidated. The present study has shown that direct intracerebroventricular injection of BFP enhances the stimulated DA release from amygdala in female specific and extra-ovary mechanism. The exact mechanism by which BFP exerts its effect on DA neurons awaits further investigation. Further studies will be needed in order to explore whether BFP could have beneficial effects for sufferers of Parkinson's disease, a disease that manifests with damaged dopaminergic tissue and impaired dopaminergic functions.

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