Korean J. of Pharmacology
Vol.28, No.2, pp.137-146, 1992

Effects of Intracerebroventricular TFMPP on Rabbit Renal Function

Young Chai Lim, Johng Bom Choi, Kyung Keun Kim and Young Johng Kook

Department of Pharmacology, Chonnam University Medical School, Kwangju, 501-190, Korea

ABSTRACT

The central tryptaminergic system has been shown to play an important role in the regulation of renal function: 5-HT_1 receptor mediate diuresis and natriuresis, whereas both 5-HT_2 and 5-HT_3 mediate antidiuresis and antinatriuresis. Recently, 5-HT_1 receptors are further subdivided into many subtypes, and central 5-HT_{1A} subtype was shown to mediate diuretic and natriuretic effects. The present study was undertaken to delineate the role of 5-HT_{1B} subtype. Trifluoromethylphenylpiperazine (TFMPP), a selective 5-HT_{1B} agonist in doses ranging from 8 to 750 μg/kg icv elicited diuresis, natriuresis and kaliuresis in dose-dependent fashion, with the fractional excretion of filtered Na reaching 5.44% with 250 μg/kg icv. The natriuresis outlasted the transient increases in renal hemodynamics, suggesting humoral mediation in the decreased tubular Na reabsorption. Plasma concentration of atrial natriuretic peptide increased along with the natriuresis. Systemic blood pressure transiently increased. When given intravenously, no diuresis and natriuresis was elicited, indicating the central mechanism. The icv TFMPP effects were not significantly affected by icv methysergide, a nonselective 5-HT_1 blocker. Both ketanserin and MDL 72222, selective 5-HT_2 and 5-HT_3 antagonists, resp., did not abolish the TFMPP effects. Nor did NAN-190, 5-HT_{1A} blocker, affect the TFMPP effects. These observations suggest that central 5-HT_{1B} receptors may play a role in the central regulation of renal function by exerting diuretic and natriuretic influences, mainly through natriuretic factors.

Key Words: Renal Function, Trifluoromethylphenylpiperazine, Central 5-HT_{1B} subtype, Atrial natriuretic peptide

INTRODUCTION

Various endogenous biogenic amines in the central nervous system are involved in the central regulation of renal function, and the tryptaminergic system is also shown to take part in it. 5-Hydroxytryptamine (5-HT), when given icv in the rabbit, elicits diuresis and natriuresis (Park, 1972; Kook *et al.*, 1988). Kook *et al.* (1988) observed that the renal effects of 5-HT is brought about mainly by inhibition of tubular sodium reabsorption through humoral natriuretic factors and that methysergide, a 5-HT blocker, can antagonize the icv 5-HT effects, thus indicating that 5-HT receptors mediate the effects. On the other hand, ketanserin, a selective 5-HT_2 antagonist, which by itself does not affect the renal function, augmented the natriuretic action of icv 5-HT (Kook *et al.*, 1990). Based on these findings, a hypothesis has been put forward that central 5-HT_1 receptors me-

diate the diuretic and natriuretic effects, whereas 5-HT_2 receptors exert opposite effects (Kook *et al.*, 1990). In addition, Kook *et al.* (1991) reported that central 5-HT_3 receptors also have antidiuretic influence upon kidney, based on the observations made with selective 5-HT_3 agonists and antagonists.

On the other hand, 5-HT receptors are not homogeneous and may consist of at least three major subtypes, 5-HT_1, 5-HT_2 and 5-HT_3 (Bradley *et al.*, 1985). Particularly, 5-HT_1 receptors are further subdivdided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} subtypes, etc (Bradley *et al.*, 1985; Hartig, 1989; Gothert and Schlicker, 1990). The characteristics of each subtype are being elucidated, as specific agonists and antagonists for each subtype are becoming available. As for the 5-HT_{1A} receptor, diuretic role was suggested on the basis of observations using 8-OH-DPAT and PAPP, selective 5-HT_{1A} agonists (Kim, 1990; Yang, 1991). However, little information is available as for other subtypes including 5-HT_{1B}. Therefore, we attempted in this study to delineate the role of central 5-HT_{1B} receptors by observing the renal effects of icv TFMPP, a specific 5-HT_{1B} agonist.

METHODS

Adult rabbits of either sex, 1.8~2.4 kg, were anesthetized with 1g/kg urethane s.c. Airway was kept free with a T-tube inserted into the trachea. Infusion of 0.3% NaCl and 3% glucose solution containing 45 mg% of para-amino-hippuric acid and 250 mg% of creatinine was given into an ear vein at a rate of 0.5 ml/min. Through a small midline incision close to the symphysis, both ureters were cannulated with PE tubings for the collection of urine samples, and for sampling blood specimens a femoral artery was cannulated with PE tubing, which was kept patent with heparin-saline (400 U/ml). For intracerebroventricular (icv) administration of the agents a lateral ventricle of the cerebrum was cannulated. A hole was drilled on the skull at a point 1.5 cm rostral to the occipital tubercle and 0.5 cm lateral to the midline, and a cannula made of PE tubing of 1.5 mm O.D. was introduced obliquely until clear cerebrospinal fluid appeared in the cannula, and then it was kept in place by cementing to the bone. The volume administered did not exceed 0.15 ml. At the end of each experiment the location of the cannula tip was checked by dissection.

When urine flow rate (UFR) became stable several hours after the initiation of the infusion, collection of clearance samples was started. After two 10-minute clearance periods the agent was administered, and then two 10-min and three 20-min clearance samples were collected. The blood samples were obtained at midpoint of each clearance period from a femoral artery and were immediately centrifuged to separate the plasma.

Quantitative analyses of creatinine were done by the method of Phillips (1944) and PAH by that of Smith *et al.* (1945). Na and K concentrations were determined by flamephotometry, and the osmolality with osmometer. Atrial natriuretic peptide (ANP) was determined by radioimmunoassay as described by Cho *et al.* (1989). Blood samples were collected in cold tubes containing ethylenediaminetetraacetic acid, phenylmethylsulfonyl fluoride, soybean trypsin inhibitor and aprotinin. One ml of plasma was passed through Sep-pak C_{18} cartridge, washed with 4 ml of 0.1% trifluoroacetic acid (TFA), and eluted with 2 ml of 60% acetonitrile in TFA. The eluant was dried with a Speedvac evaporator. The lyophilized samples were reconstituted with 100 μl Tris acetate buffer, added with 100 μl antiserum (for AP III) and incubated at 24℃ for 18~24 hrs, and then after adding ^{125}I-AP III, incubated another 24 hrs. Bound-form was separated from free-form using double antibody. And the radioactivity was measured with a gamma counter.

Statistical significance was assessed either with Student's t-test or with ANOVA with repeated measures on time (Winer, 1971). If significant differences were detected with ANOVA, further analyses as required were performed to determine which of the groups differed from the appropriate controls. For multiple group comparison Bonferroni's modified t-test was applied (Wallenstein *et al.*, 1980).

TFMPP (m-trifluoromethylphenylpiperazine) hydrochloride, MDL 72222 (3-tropanyl-3, 5-dichlorobenzoate), NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimido)-butyl] piperazine) hydrobromide,

and S(−)-propranolol were obtained from Research Biochemicals Inc., Methysergide from Sandoz Inc., and ketaserin from Janssen Inc. They were dissolved in 0.9% NaCl solution immediately before administration. Doses were calculated as the base.

RESULTS

Renal effects of intracerebroventricular TFMPP

When 8 μg (=30 nmoles)/kg of TFMPP was administered icv, transient increases in urine flow rate (UFR) and Na excretion were observed immediately after administration. Systemic blood pressure showed no changes.

Increasing the doses three-fold to 25 μg (=100 nmoles)/kg produced greater changes in renal function. During the two 10-min periods immediately following administration, UFR and Na excretion significantly increased. Glomerular filtration rate (GFR, =Ccr) also increased in the first 10-min period and then returned to the control level. Free water reabsorption did not change significantly and nor did the systemic arterial pressure.

In rabbits which received 80 μg (=300 nmoles)/kg, the renal responses became more prominent, as shown in Fig. 1. UFR increased about 2.2 times the control value in the first 10-min period. Sodium excretion and fractional excretion of sodium (FE_{Na}) also increased markedly in the period. Renal hemodynamics increased transiently and decreased in the next period. UFR tended to return to the control level in the 2nd 10-min period. However, the natriuretic effects lasted until 40 min after the administration. Increases in potassium excretion were also noted. Mean arterial pressure showed transient elevation for the first 10-min period.

With the dose further increased to 250 μg (=1 μ moles)/kg icv, the diuretic, natriuretic and kaliuretic effects became more intensified. UFR significantly increased up to 2.8 times the control level at its peak during the two 10-min periods after administration. Renal plasma flow ($=C_{PAH}$) and GFR also increased by 35% and 40%, respectively, in the first 10-min peirod and returned to the control level in the next. Immediately after administration Na excretion and FE_{Na} increased more than 5 times the control value, and the natriuresis was maintained up to 40 min after administration, outlasting the changes in renal hemodynamics. Transient increase in systemic blood pressure was observed right after administration.

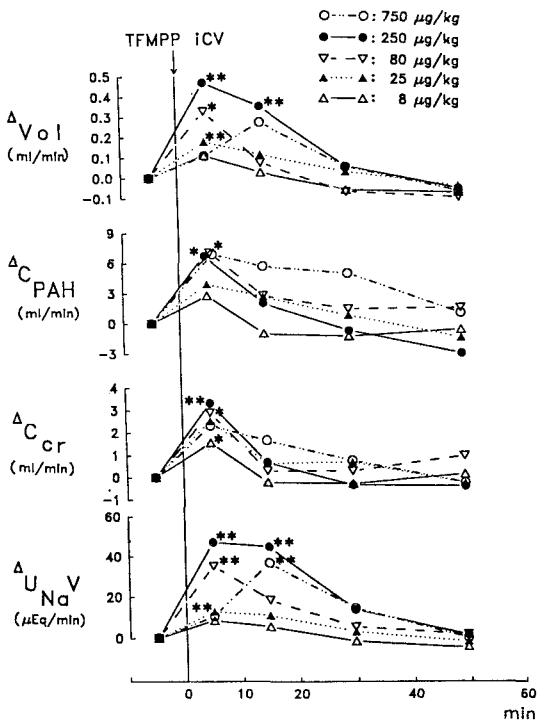

Fig. 1. Influence of icv TFMPP 8~750 μg/kg on rabbit renal function. Mean changes from the control values with one S.E. are shown. Abbreviations: Vol, rate of urine flow; C_{PAH} and C_{cr}, clearances of PAH and creatinine, resp; $U_{Na}V$, excretory rates of sodium. Asterisks indicate significant changes from the control value. *=P<0.05; **=P<0.01.

Further increase of doses up to 750 μg (=3 μ moles)/kg resulted also in greater diuresis and natriuresis. Systemic blood pressure increased by 38.5 mmHg right after administration and then tended to decline. Fig. 1 depicts the changes of renal function induced by various doses of TFMPP.

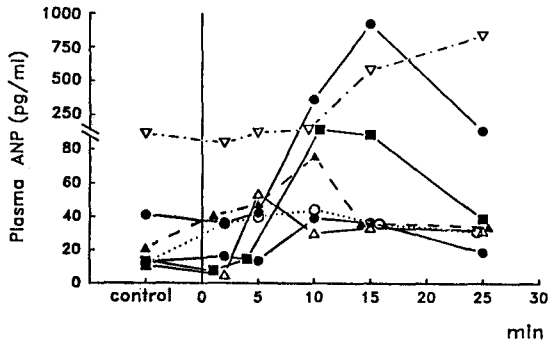

Fig. 2. Changes of plasma concentration of atrial natriuretic peptide by icv administration of TFMPP 250 μg/kg.

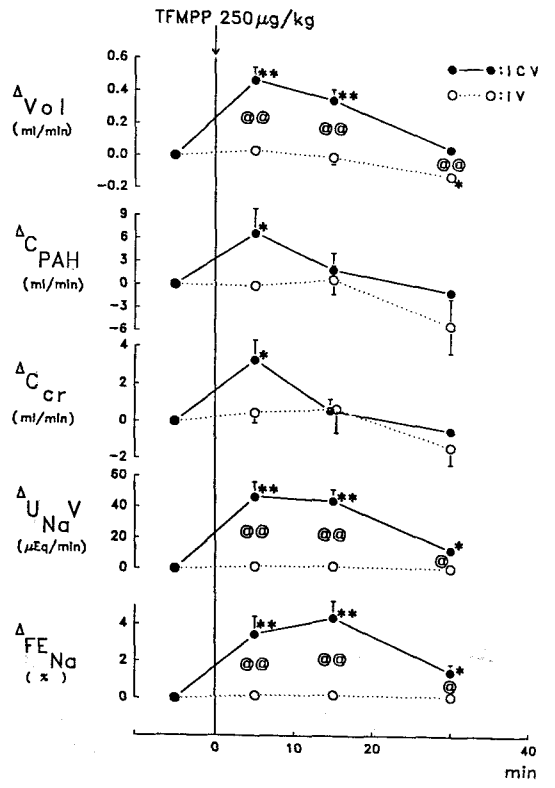

Fig. 3. Effects of icv and intravenous (iv) TFMPP 250 μg/kg on rabbit renal function. Other legends as in Fig. 1.

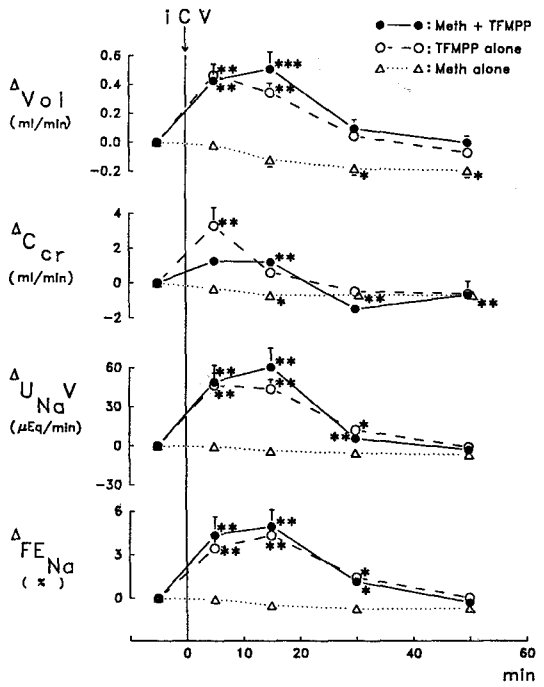

Fig. 4. Influence of methysergide (Meth) 40 μg/kg icv on the icv TFMPP effects. Methysergide was administered 3 min before 250 μg/kg TFMPP.

Influence of TFMPP on the plasma concentration of ANP

As the natriuresis outlasting the hemodynamic changes suggested the involvement of some humoral natriuretic factor, the plasma concentration of ANP was determined. A dose of 250 μg/kg TFMPP was chosen. As shown in Fig. 2, the ANP level increased in all 7 cases, reaching the peak value of 220.4±124.6 pg/ml at 15 min from the control value of 38.1±20.0 pg/ml.

Renal effects of intravenous TFMPP

To test the possibility that the agent given icv might have leaked out of the icv injection site into the systemic circulation and thus have affected the renal function directly, the renal effects of intravenously given TFMPP were observed in doses of 250 μg/kg, which exerts maximal natriuretic effects when given icv. As clearly

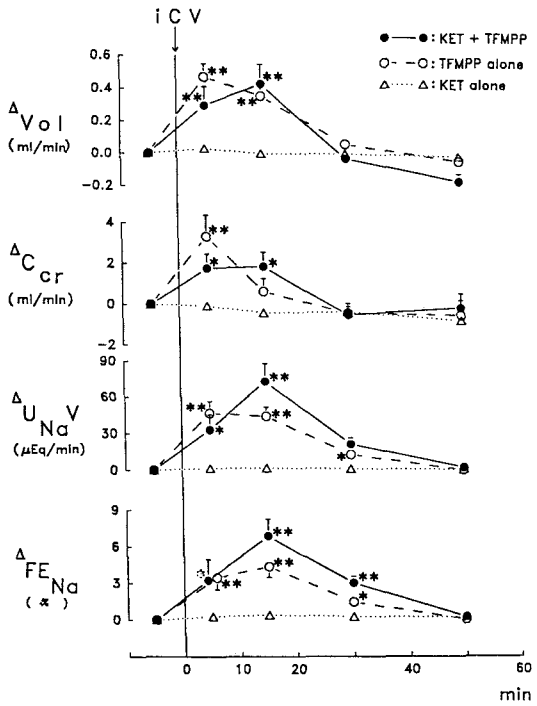

Fig. 5. Influence of ketanserin (KET) 40 μg/kg icv on the icv TFMPP effects. Ketanserin was administered 3 min before 250 μg/kg TFMPP.

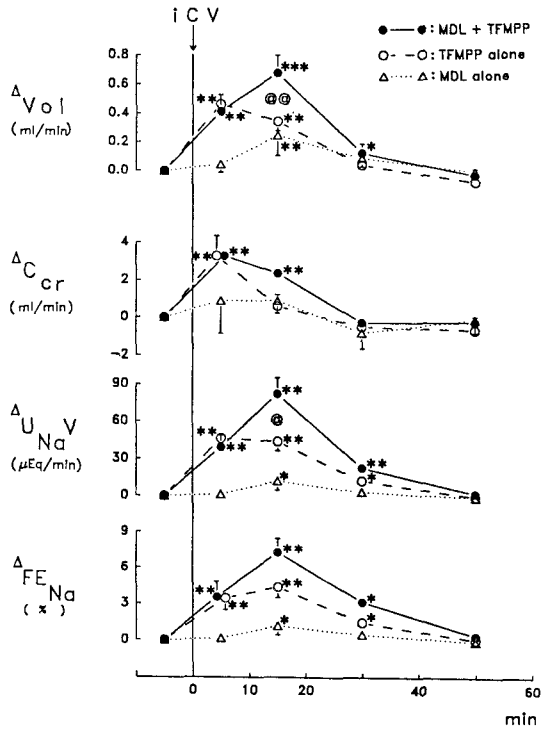

Fig. 6. Influence of MDL 72222 (MDL) 30 μg/kg icv on the icv TFMPP effects. MDL 72222 was administered 3 min before 250 μg/kg TFMPP. Significant differences between TFMPP alone group and MDL+TFMPP group were marked with ⓐ =P<0.05; ⓐⓐ =P<0.01.

shown in Fig. 3, no significant changes in renal function were noted when given iv, thus differing significantly from the icv group. Also, systemic blood pressure did not show any significant changes, unlike the elevation observed when given icv.

Influence of antagonists on the TFMPP effects

First, the effects of methysergide, a 5-HT receptor antagonist, on the renal action of icv TFMPP were tested (Fig. 4). 40 μg/kg methysergide icv alone showed decreasing tendency in all parameters of renal function except systemic blood pressure. Even after pretreatment with methysergide icv, TFMPP 250 μg/kg produced significant diuretic and natriuretic effects, not differing from the control group without the pretreatment. Rather, the pretreatment tended to augment the TFMPP effects.

Next, the influence of ketanserin, a specific 5-HT_2 receptor antagonist, on the effects of icv TFMPP was observed (Fig. 5). Ketanserin 40 μg/kg icv did not markedly affect the renal function. When TFMPP 250 μg/kg were administered 3 min after ketanserin, UFR, Na excretion and FE_{Na} increased significantly. Increases in Na excretion and FE_{Na} were sustained even in the 20~40 min period, in which renal hemodynamics declined below the control level. Systemic blood pressure also showed transient increase immediately after adminis-tration like the TFMPP-alone group.

MDL 72222 (MDL), a selective 5-HT_3 receptor blocker, 30 μg/kg icv produced significant diuresis and natriuresis in the second 10-min period and rapid recovery to the control level followed. After pretreatment with MDL, icv TFMPP 250 μg/kg elicited significantly greater natriuresis and di-

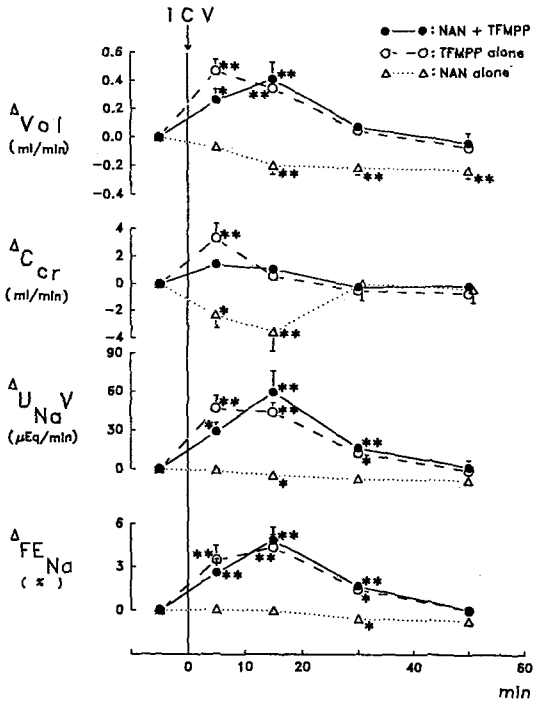

Fig. 7. Influence of NAN-190 (NAN) 40 μg/kg icv on the icv TFMPP effects. NAN-190 was administered 3 min before 250 μg/kg TFMPP.

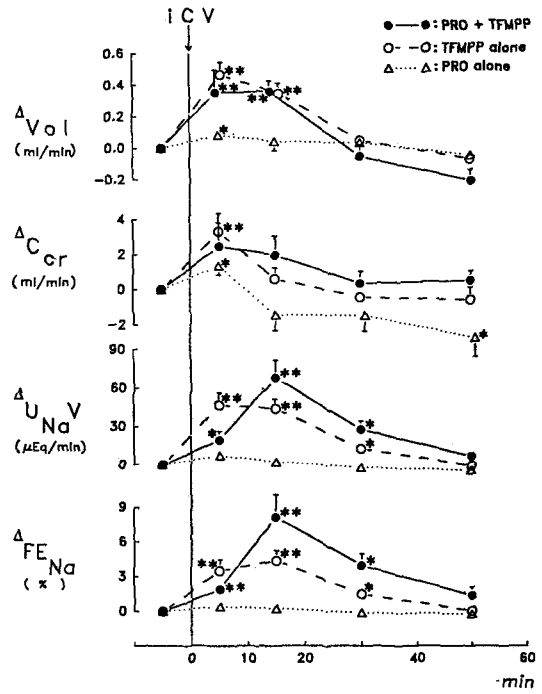

Fig. 8. Influence of S(−)-propranolol (PRO) 25 μg/kg icv on the icv TFMPP effects. S(−)-propranolol was administered 3 min before 250 μg/kg TFMPP.

uresis in the 10~20 min period, compared with the TFMPP-alone group (Fig. 6).

Next, the effect of NAN-190 (NAN), a recently introduced 5-HT_{1A} blocker, on TFMPP action was observed. NAN 40 μg/kg icv reduced UFR and Na excretion, along with the decreases of renal hemodynamics and arterial blood pressure. When 250 μg/kg TFMPP was given 3 min after NAN administration, TFMPP effects were not influenced by NAN (Fig. 7). The influence of S(−)-propranolol, beta-adrenoceptor blocker with some 5-HT_{1B} antagonizing properties, on the TFMPP effects was assessed inasmuch as no selective 5-HT_{1B} antagonist is known up to the present. S(−)-propranolol alone showed transient and slight increase in UFR following icv administration. Even after pretreatment with S(−)-propranolol, the natriuretic and diuretic responses of icv TFMPP were not affected (Fig. 8).

DISCUSSION

The heterogeniety of 5-HT receptors, as first suggested by Gaddum and Picarreli (1957) has been substantiated by radioligand binding studies (Peroutka and Snyder, 1979). Further studies led to a classification of 5-HT receptors into 3 subtypes (Bradley *et al.*, 1985). Heterogeniety of 5-HT_1 receptors was first demonstrated in 1981 by Pedigo *et al.*, who subdivided 5-HT_1 receptors further into 5-HT_{1A} and 5-HT_{1B} subtypes, the former being inhibted by spiperone, whereas the latter not affected by it. Later, 5-HT_{1C} subtype which has high affinity for mesulerzine was identified by autoradiography and binding studies (Pazos *et al.*, 1984). In addition, 5-HT_{1D} sites was also known as another subtype of 5-HT_1 receptors (Waeber *et al.*, 1989a: Waeber *et al.*, 1989b).

Physiological roles of 5-HT_2 and 5-HT_3 receptors have been fairly well defined thanks to the introduction of potent selective agonists and antagonists for those receptors. In case of 5-HT_1 subtypes, however, few specific agents have so far been introduced in spite of extensive search for them, thus hindering the clear characterization of those receptors.

Regarding the role of central tryptaminergic system in the regulation of renal function, both 5-HT_2 and 5-HT_3 receptors have been shown to mediate antidiuretic and antinatriuretic action as evidenced by the renal effects of specific agonists and antagonists (Kook *et al.*, 1990; Kook *et al.*, 1991; Kim, 1991; Kim, 1992). As for 5-HT_1 subtypes, they are suggested to mediate diuretic influence (Kim, 1990; Yang, 1991). However, few data on the role of 5-HT_{1B} receptor is available.

In this study, icv TFMPP, a selective 5-HT_{1B} agonist, dose-relatedly increased urine flow rate for 20 min and urinary Na excretion for 40 min after administration. Renal hemodynamics, however, showed significant increase only in the first 10-min period after administration and returned to the control level in the next 10-min period. This suggests that diuretic and natriuretic effects of icv TFMPP was brought about mainly through decreased tubular Na reabsorption via humoral natriuretic factors although the transient improvement of renal hemodynamics may also have contributed to the natriuresis to a certain degree.

Among humoral agents which produce natriuresis, atrial natriuretic peptide (ANP) was first identified (DeBold *et al.*, 1981) and shown to be present also in brain (Tanaka *et al.*, 1984). Discovery of brain natriuretic peptide (BNP) followed (Sudoh *et al.*, 1988). Recently, C-type natriuretic peptide (CNP) was identified in porcine brain (Sudoh *et al.*, 1990). All these three peptides have amino acid sequence quite similar to each other and all are involved in the regulation of body fluids, electrolytes and blood pressure. It is assumed that CNP in particular might play an important role in central control of cardiovascular function, for the concentration of CNP is the highest among those three peptides in CNS (Furuya *et al.*, 1990), although its potency in producing natiuretic, diuretic and hypotensive response is less than those of ANP and BNP (Sudoh *et al.*, 1988; Sudoh *et al.*, 1990). Increases in the plasma concentration of ANP, the most potent natriuretic humoral factor, as revealed in this study, accompanied the natriuresis of icv TFMPP. From the control level of 38.1 pg/ml, ANP began to increase immediately after drug administration and reached to a peak value of 220.4 pg/ml, 6 times the control value, between 13 to 16 min after administration. Thus ANP is evidently involved in the icv TFMPP action. However, its origin and release mechanism are yet to be determined. Also, to be clarified further are the influences of TFMPP icv on the level of BNP and CNP. The fact that kaliuresis by icv TFMPP is produced together with natriuresis may suggest that inhibition of Na reabsorption takes place chiefly in the proximal portion of the tubules.

It is noteworthy that the diuretic and natriuretic effects of icv TFMPP were so strong that FE_{Na} increased in its peak up to 5.5 times the control value, with the maximal differences from the control of Na excretion and UFR reaching 46.63 mEq /L and 0.467 ml/min, respectively. The fractional Na excretion reached 5.44%, a value comparable to the effects of major diuretics, such as acetazolamide or thiazides. Central 5-HT_1 receptor, in contrast to the other two types, has been shown to mediate diuresis and natriuresis and it was further suggested that 5-HT_{1A} and 5-HT_{1B} subtypes among 5-HT_1 receptor subtypes are involved in such action. Our present study suggests that 5-HT_{1B} subtype might play a major role in the center-mediated natriuresis.

Methysergide pretreatment, contrary to our expectation, showed increase in natriuresis. Methysergide has been introduced initially as 5-HT_1 blocker, but it was later shown to have affinity also for 5-HT_2 receptor, raising suspicion as to its selectivity. Therefore, it might be possible to postulate that the augmentation of the icv TFMPP effects after methysergide pretreatment resulted from antagonizing the possible antidiuretic influence through 5-HT_2 receptors.

Both ketanserin and MDL 72222 also did not abolish the TFMPP effects, indicating that 5-HT_2 and 5-HT_3 receptors are not involved in the icv TFMPP-induced natriuresis. After pretreatment of MDL, the icv TFMPP responses were significantly intensified, which is consistent with the re-

port of Kook *et al.* (1991) that central 5-HT_3 receptors may exert antidiuretic influence. Although 5-HT_3 receptor might also be possibly affected by TFMPP, the diuretic and natriuretic effects of TFMPP were not abolished by MDL pretreatment, thus rendering such possibility unlikely.

NAN-190 (NAN), a newly introduced 5-HT_{1A} antagonist, also did not affect the TFMPP responses, suggesting that 5-HT_{1A} receptors is not related to the TFMPP effects. However, it has been recently demonstrated that the hypothermia and secretion of hydrocortisone induced by specific 5-HT_{1A} agonists are not blocked by NAN, although NAN can antagonize the behavioral action such as forepaw treading induced by the same agonist (Przegalinski *et al.*, 1990). In addition, NAN did not abolish the natriuresis by specific agonists such as 8-OH-DPAT and PAPP (Yang, 1991; Jeong, 1991). Therefore, the possibility that 5-HT_{1A} is also involved in the TFMPP effect cannot be readily ruled out and the advent of more specific antagonists are awaited.

5-HT_{1B} receptors have been identified in the rat and mouse brain, in a renal epithelial cell line from the opossum, and in fibroblasts from the hamster (Peroutka, 1988; Hoyer, 1988; Murphy and Byland, 1989). 5-HT_{1B} binding sites in rat brain are present at high density in extrapyramidal areas, such as substantia nigra and the globus pallidum (Seuwen *et al.*, 1989). 5-HT_{1B} receptors are negatively coupled to adenylate cyclase and are thought to mediate inhibition of neurotransmitter release as presynaptic receptors (Maura and Raiter, 1986; Starke *et al.*, 1989). Selective 5-HT_{1B} antagonist is not available at present. S(−)-propranolol, a beta-adrenoceptor blocker, was found to possess some 5-HT_{1B} antagonizing properties (Pazos *et al.*, 1985a; Middlemiss, 1986). The TFMPP effect was not affected by it. However, this fact should not be taken as evidence against the involvement of 5-HT_{1B} receptor in the TFMPP effect, for the selectivity of S(−)-propranolol for 5-HT_{1B} is seriously in doubt. For example, in drug discrimination study, generalization of TFMPP stimulus was not blocked by S(−)-propranolol pretreatment (Glennon *et al.*, 1987), although S(−)-propranolol has some antagonizing activities in other models of experiments. Therefore, again, the advent of more specific antagonists of 5-HT_{1B} will settle the problem.

Regarding 5-HT_{1C} receptors, it has been described that they are present primarily at choroid plexus and are involved in the regulation of volume and composition of CSF (Davson, 1967), providing some possibilities of involvement of 5-HT_{1C} receptor in the TFMPP effects. However, 5-HT_{1C} binding site occupies only 10% of all 5-HT_1 binding sites which are labeled by [^{3}H]-5-HT (Pazos, 1985b) and a lesser role is assumed for 5-HT_{1C} receptor than that of 5-HT_{1B} receptor.

The presence of 5-HT_{1D} binding sites could be identified in binding studies by the observation that [^{3}H]-5-HT still could be labeled even after pretreatment of antagonists of all other 5-HT_1 receptors subtypes. 5-HT_{1D} binding sites are detected in the brain of species such as guinea-pig, pig and calf, in which 5-HT_{1B} sites are not found (Waeber *et al.*, 1989a; Waeber *et al.*, 1989b). The distribution of 5-HT_{1D} sites is similar to that of 5-HT_{1B} receptors in the rat brain and 5-HT_{1D} receptors also inhibit the activity of adenylate cyclase, suggesting the evolutionary correlation between the two receptors (Schoeffter *et al.*, 1988). Therefore it is possible that the TFMPP effects might be mediated rather by 5-HT_{1D} receptors, not by 5-HT_{1B} receptors. However, no report is avilable regarding the identification of 5-HT_{1D} receptors in the rabbit brain. The possible involvement of 5-HT_{1D} receptor in the TFMPP effects should be clarified by specific 5-HT_{1D} agonists and antagonists, not available yet.

REFERENCES

Bradley PB, Engel G, Feniuk W, Fozard JR, Hamphrey PPA, Middlemiss DN, Mylecharene EJ, Richardson BP and Saxenal PR: *Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacol 25: 563-575, 1985*

Cho KW, Seul KH, Kim SH, Kyu H, Seul KM and Koh GY: *Epicardial release of immunoreactive atrial natriuretic peptide in the heart. Gen Comp Endocr 74: 127-135, 1989*

Davson H: *The physiology of the cerebrospinal fluid. 1967 (Little Brown, Boston MA)*

DeBold AJ, Borenstein, HB, Veress AT and Sonnenberg

H: *A raid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci 28: 89-94, 1981*

Furya M, Takehisa M, Minamitake Y, Kitajima Y, Hayashi Y, Ohnama N, Ishihara T, Minamino N, Kangawa K and Matsuo H: *Novel natriuretic peptide, CNP, potently stimulates cyclic GMP production in rat cultured vascular smooth muscle cells. Biochem Biophys Res Commun 170: 201-208, 1990*

Gaddum JH and Picarelli ZP: *Two kinds of tryptamine receptor. Brit J Pharmacol Chemother 12: 323-328, 1975*

Glennon RA, Pierson ME and McKenney JD: *Stimulus generalization of 1-(3-trifluoromethylphenyl)-piperazine (TFMPP) to propranol and mesulergine, Pharmacol Biochem Behav 29: 197-199, 1987*

Gothert M and Schlicker E: *Identification and clarification of $5HT_1$ receptor subtypes. J Cardiovasc Pharmacol 15 (Suppl 7): S1-S7, 1990*

Hartig PR: *Molecular biology of 5-HT receptors. Trends Pharmacol Sci 10: 64-69, 1989*

Hoyer D: *Functional correlates of serotonin 5-HI_1 recognition. J Recept Res 8: 59-81, 1988*

Jeong YH: *Influence of intracerebroventricular NAN-190 on rabbit renal function. Inaug Dissert Chonnam Univ 1991*

Kim C: *Renal effects of intracerebroventricular DOI, a 5-HT_2 agonist, in rabbits. Inaug Dissert Chonnam Univ 1992*

Kim DM: *Effects of intracerebroventricular phenylbiguanide on rabbit renal function. Inaug Dissert Chonnam Univ 1991*

Kim KS: *Renal effects of intracerebroventricular PAPP in the rabbit. Inaug Dissert Chonnam Univ 1990*

Kook YJ, Kim KK, Min JS, Lim YC and Kook H: *Studies on tryptaminergic regulation of rabbit renal function. Chonnam J Med Sci l: 139-147, 1988*

Kook YJ, Kim KK, Kim YN, Lim YC and Kook H: *Influence of intracerebroventricular ketanserin on rabbit renal function. Korean J Pharmacol 26: 153-159, 1990*

Kook YJ, Lim YC, Kim KK, Kook H and Oh BC: *Role of central 5-HT_3 recepotrs in regulation of rabbit renal function. Chonnam J Med Sci 4: 27-39, 1991*

Maura G and Raiteri M: *Cholinergic terminals in rat hippocampus possess 5-HT_{1B} receptors mediating inhibition of acetylcholine release. Eur J Pharmacol 129: 333-337, 1986*

Middlemiss DN: *Stereoselective blockade at [^{3}H]-5HT binding sites and at the 5-HT autoreceptor by propranolol. Eur J Pharmacol 101: 289-293, 1984*

Murphy TJ and Bylund DB: *Characterization of serotonin 1B receptors negatively coupled to adenylate cyclase in OK cells, a renal epithelial cell line from the opossum. J Pharmacol Exp Ther 249: 535-543, 1989*

Park IK: *Influence of intraventricular 5-hydroxytryptamine on renal function of the rabbit. Chonnam Med J 9: 33-42, 1972*

Pazos A, Engel G and Palacios JM: *Adrenoceptor blocking agents recognize a subpopulation of serotonin receptors in brain. Brain Res 343: 403-408, 1985a*

Pazos A, Hoyer D and Palacios JM: *The binding of serotonergic ligands to porcine choroid plexus characterization of a new type of serotonin recognition site. Eur J Pharmacol 106: 539-546, 1984*

Pazos A and Palacios JM: *Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-l receptors. Brain Res 346: 205-230, 1985b*

Pedigo NW, Yamamura HI and Nelson DL: *Discrimination of multiple ^{3}H-5-hydroxytryptamine binding sites by the neuroleptic spiroperidol in rat brain. J Neurochem 36: 220-226, 1981*

Peroutka SJ and Snyder SH: *Multiple serotonin receptors: Differential binding of ^{3}H-serotonin, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol. Mol Pharmacol 16: 687-699, 1979*

Perouka SJ: *5-Hydroxytryptamine receptor subtypes. Annu Rev Neurosci 11: 45-60, 1988*

Phillips RA: *In, Quantitative Clinical Chemistry. Vol 2, Methods, Peters & Van Slyke (Eds), Williams & Wilkins, 1944*

Przegalinski E, Ismaiel AM, Chojnacka-Wojcik E, Budziszewska B, Tatarczynska E and Blaszczynska E: *The behavioral, but not the hypothermic or corticosterone response to 8-hydroxy-2(-di-n-propylamino)-tetralin, is antagonized by NAN-190 in the rat. Neuropharmacol 29: 521-526, 1990*

Schoeffter P, Waeber C, Palacios JM and Hoyer D: *The 5-hydroxytryptamine 5-HT_{1D} receptor subtype is negatively coupled to adenylate cyclase in calf substantia nigra. Naunyn-Schmiedeberg's Arch Pharmacol 337: 602-608, 1988*

Seuwen K, Magnaldo I and Pouyssegur J: *Serotonin stimulates DNA synthesis in fibroblasts acting through 5-HT_{1B} receptor coupled to a G_i protein. Nature 335: 254-254, 1988*

Smith HW, Finkelstein N, Aliminosa L, Crawford B and Graber B: *The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J Clin Invest 24: 388-404, 1945*

Starke K, Gothert M and Kilbinger H: *Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol Rev 69: 864-989, 1989*

Sudoh T, Kangawa K, Minamino NO and Matsuo H: *A new natriuretic peptide in porcine brain. Nature 332:*

78-80, 1988

Sudoh T, Minamino N, Kangawa K and Matsuo H: *C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. Biochem Biophys Res Commun 168: 863-870, 1990*

Tanaka I, Misno KS and Inagami T: *Atrial natriuretic factor in rat hypothalamus, atria and plasma: determination by specific radioimmunoassay. Biochem Biophys Res Commun 124: 663-668, 1984*

Waeber C, Schoeffter P, Palacios JM and Hoyer D: *5-HT_{1D} receptors on guinea-pig and pigeon: radioligand binding and biochemical studies. Naunyn-Schmiedeberg's Arch Pharmacol 340: 479-485, 1989a*

Waeber C, Pietl MM, Hoyer D and Palacios JM: *5-HT_{1D} receptors in the vertebrate brain: regional distribution examined by autoradiography. Naunyn-Schmiedeberg's Arch Pharmacol 340: 486-494, 1989b*

Wallenstein S, Zucker CL and Fleiss JL: *Some statistical methods used in circulation research. Circ Res 47: 1-9, 1980*

Winer BJ: *Statistical Principles in Experimental Design. 2nd ed. McGraw-Hill, New York, 1971*

Yang DH: *Renal effects of intracerebroventricular 8-OH-DPAT in the rabbit. Inaug Dissert Chonnam Univ 1991*

=국문초록=

뇌실내 TFMPP가 가토신장기능에 미치는 효과

전남대학교 의과대학 약리학교실

임영채 · 최종범 · 김경근 · 국영종

신장기능조절에 있어서 중추 tryptamine계가 관련되어 있으며, 5-HT_1수용체는 이뇨적인 역할을 하고 있는 반면에 5-HT_2 및 5-HT_3수용체는 항이뇨적인 영향을 미치고 있음이 밝혀진 바 있다. 또한 5-HT_1수용체도 단일하지 않고 여러 subtype가 존재함이 알려져 있다. 5-HT_{1A}수용체의 역할에 관해서는 신기능에 이뇨적인 영향을 미치고 있음이 시사된 바 있다. 본 연구에서는 중추 tryptamine성 신기능 조절에 있어서 5-HT_{1B}수용체의 역할을 구명하고자 하였다.

선택적 5-HT_{1B} agonist인 TFMPP 8~750 μg/kg을 가토 측뇌실내로 투여하면 투여량에 비례하여 이뇨 및 Na과 K 배설의 증가를 초래하였으며, 250 μg/kg 투여시에는 Na의 배설 분획이 5.44 %까지 증가하였다. Na배설 촉진작용은 신혈류역학의 증가 보다도 훨씬 지속하여, 세뇨관에서의 Na재흡수 감소작용이 체액성 기전임을 시사하였다. TFMPP 250 μg/kg icv투여시에 natriuresis와 함께 혈장내 atrial natriuretic peptide 농도가 약 6배 증가되었다. TFMPP 250 μg/kg을 정맥내로 투여하였을때는 뇌실내 투여시와는 상이하게 신기능에 별다른 유의한 변동을 초래하지 않았다. 이와같은 TFMPP의 diuresis 및 natriuresis는 각각 5-HT_2 및 5-HT_3 수용체의 선택적 antagonist인 ketanserin과 MDL 72222의 전처치에 의하여 차단되지 않았으며, methysergide에 의해서도 억제되지 않았다. 또한 5-HT_{1A} antagonist로 알려진 NAN-190도 TFMPP의 작용을 차단하지 못하였으며 S(−)-propranolol도 영향을 미치지 않았다.

본 연구의 결과 중추 5-HT_{1B}수용체는 신장기능에 이뇨 및 Na배설 촉진적인 영향을 미치고 있고 이작용에 atrial natriuretic peptide가 관여함을 알 수 있었다.