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Effects of Fusaric (5-butylpicolinic) Acid on the Monosynaptic Reflex Neural Activity of Cat Spinal Cord

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It has been demonstrated that most hypertensive drugs which cause increases in levels of norepinephrine influence the stimulation of monosynaptic reflex (MSR) neural activity. However this report discusses the effects of a hypotensive drug, which causes decreases in levels of norepinephrine, on the MSR amplitude of acute spinal cats. This drug is 5-butylpicolinic acid (fusaric acid: FA) which is an effective hypotensive agent and a potent inhibitor of dopamine β-hydroxylase (approximately 10 times more potent than disurfiram). Intravenous injections of FA increased MSR neural activity in a dose-dependent manner. The FA-induced neural activity was gradually depressed by treatment with haloperidol, a dopamine and/or a α receptor blocker and methysergide, a serotonin receptor blocker, respcetively. In addition, this neural activity was potentiated by the sequential administration of L-dopa. FA did not cause increases in the blood pressure but inhibit the synthesis of norepinephrine from dopamine. These results suggest that not only hypertensive but also hypotensive drugs can affect the increase of MSR neural activity, and dopamine plays an important role in FA-induced neural activity.

(Key Words: Fusaric Acid, Monosynaptic Reflex, Neural Activity, Dopamine-β-hydroxylase, Acute Spinal Cats)

INTRODUCTION

5-Butylpicolinic acid (fusaric acid; FA) is a potent inhibitor of a purified bovine adrenal dopamine-β-hydroxylase [3, 4-dihydroxyphenylalanine, ascorbate: oxygen oxidoreductase (hydroxylating, EC 1.14.2.1)] (DBH), the enzyme which catalyzes the β-hydroxylation of dopamine (DA), the last step in the biosynthesis of norepinephrine (NE), and it is an effective hypotensive agent (9). Hidaka reported that FA did not alter the cerebral dopamine concentrations but norepinephrine and serotonin (5-HT) concentrations were changed (10). After three injections of FA (75 mg/kg) the concentration of rat cerebral 5-HT increased by 25% while that of NE decreased by 40% and there was no significant effect on MAO activity or the concentration of 5-hydroxy-indoleacetic acid, which is the end product of 5-HT, compared with control values. this increase of 5-HT suggested that it may be affected by depletion of NE. This hypothesis is supported by the following facts: (i) an increase of 5-HT is invariably correlated with depletion of NE, and (ii) increases of 5-HT after FA injection are smaller than those following administration of MAO inhibitors, a potent example of which, pargyline (75 mg/kg), doubled 5-HT concentrations, and this increase was

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not affected by FA. NE concentrations were not significantly different from control values when pargyline and FA were administered sequentially, and then the failure of FA to alter the concentration of DA suggested that DA may be degraded by O-methylation or oxidative deamination rather than by conversion to NE. It has been shown that the spinal neuronal activity is affected by catecholamines and/or 5-HT (1, 4, 6, 15, 17). Recently, we reported the effects of DA on the c-fiber reflex in acute spinalized cats (13), and the effects of peripheral catecholamines on the monosynaptic spinal reflex of cats (18). These reports suggested that NE, in both the c-fiber and the peripheral nervous systems, actually affects both neuronal activities of c-fiber and the monosynaptic reflex of spinal cord, and these effects are predominantly caused by NE. At present, it is still not clear what substances in the central nervous system primarily affect the neural activity of the monosynaptic reflex of the spinal cord. It has been demonstrated that catecholamines and indolamines exist in the spinal cord (7, 8). In this report, we examined the effects of FA, as a DBH inhibitor and hypotensive drug, on the monosynaptic spinal reflex of the acute decerebrated cat. The results obtained were caused by DA and/or 5-HT in the spinal cord and not by the hypotensive effects.

METHODS

The methods used have been previously described in detail (18). Twenty adult cats of either sex under ether anesthesia had their intercollicular level artificially respired and immobilized with gallamine triethiodide. After laminectomy, stimuli (0.05 – 0.1 msec in duration) were applied to a dorsal root at a frequency of 8/min. This stimulus was supramaximal for the monosynaptic spike. Evoked potentials were recorded from the ventral root. Drugs which increase motoneuronal excitability may manifest their effect by increasing the size of the monosynaptic spike. The amount of increase is limited by the number of motoneurons available for recruitment. In all experiments the lumbosacral cord was isolated from afferent inflow by severing all dorsal roots from L_5 to S_3 in experiments with dorsal root stimulation. The mineral oil pool bathing the spinal cord and the rectal temperatures were held constant at 37°C using a thermister probe, temperature regulator and heat lamp. Carotid blood pressure was continuously monitored and all drugs were dissolved in saline and injected into the antecubital vein via an indwelling catheter. A minimum of 3 hr elapsed between the cessation of ether anesthesia and the start of control recordings. During the control period the response of the preparation was monitored for 1 hr. Preparations showing a variability in potential size greater than ± 10% were not used. The following drugs were studied: 5-butylpicolinic acid (fusaric acid; FA) (BANYU K.K.), L-3, 4-dihydroxyphenylalanine methyl ester HCl (L-dopa) (SIGMA), haloperidol (Sernace) (DAINIPPON SEIYAKU K.K.) and methysergide bimaleate (SANDOZ).

RESULTS

1) Effects of fusaric acid on the monosynaptic reflex (Fig. 1)

The i.v. injectin of FA increased the MSR amplitude in a dose-depend-

ent manner. The minimal increase in the MSR was observed with 2 mg/kg. The i.v. injection of 2 and 5 mg/kg of FA delayed the increase in the MSR amplitude by about 10 min when compared with the injection of 10 and 25 mg/kg. Moreover, the duration of the effects of FA on the MSR was also dose-dependent and ranged from 1.5 hr for 2 mg/kg to 4 to 5 hr for 25 mg/kg. The longer duration of the larger doses may have been slightly exaggerated by their longer infusion time. Although we did not show the effect of the 50 mg/kg i.v. injection of FA in this report, it also increased both the magnitude and duration of the MSR to a greater extent than the 25 mg/kg i.v. injection. Overdoses of 10 mg/kg of FA consistently increased the MSR amplitude, but lower doses (2 or 5 mg/kg) of FA frequently maintained the control level of MSR amplitude until about 10 min after injection. In addition, the i.v. injection of 10 and 25 mg/kg of FA showed hardly any change in the blood pressure and heart rate. We frequently observed a transient reduction of blood pressure after administration of a 25 mg/kg i.v. overdose of FA. In the previous report, we noted that the i.v. injection of L-dopa changed the blood pressure and the heart rate when the dose was more than 50 mg/kg. We also observed that the blood pressure increased during NE infusion, and it was parallel to the increase of spinal activity (18). These conclusions confirmed that most hypertensive drugs which cause increases in levels of NE influence the stimulation of MSR neural activity. However, i.v. injection of FA did not increase the blood pressure but induced increases in spinal activity, and these increases were slower than those by dopa, NE or DA.

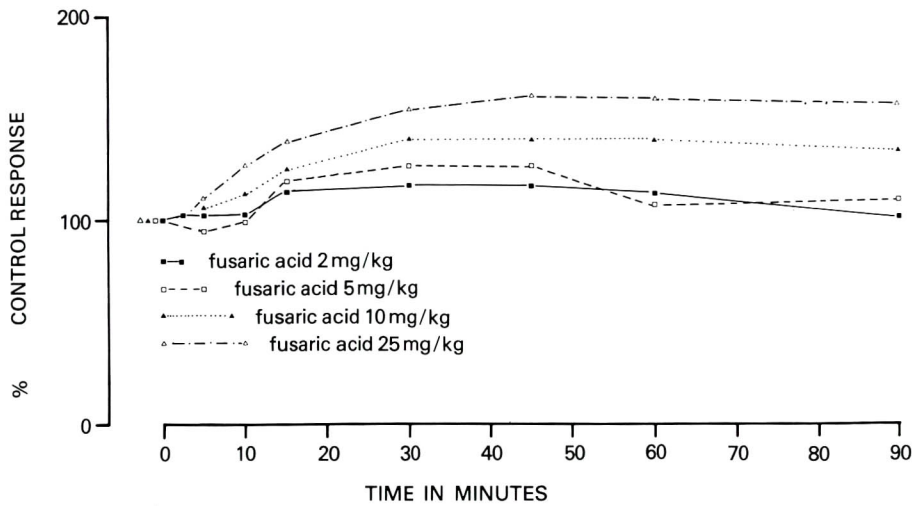

Fig. 1 Effects of different doses of FA on the monosynaptic reflex.

Each point is the average of four consecutive oscilloscope sweeps. Representative experiments with different doses of FA (2—25 mg/kg) are indicated by the line key in the lower left-hand corner. The length of the lines in the key indicates the time period during which each dose was infused. The height of the MSR just prior to the injection of FA was arbitrarily set at 100 and all subsequent measurements were reported as a percentage of this control.

2) Effects of fusaric acid followed by halopelidol on the monosynaptic reflex. (Fig. 2)

The single i.v. injection of 5mg/kg of halopelidol which is a neuroleptic drug and a DA receptor blocker, did not affect the MSR activity (18). In this experiment, the effect of FA (25mg/kg) on the MSR was strongly potentiated within 10min during the single injection of FA and this magnitude was about 75% after 1—2hr. Fig. 2 shows the inhibitory effects of halopelidol on FA-induced MSR activity. Haloperidol was administered at a dose of 1mg/kg/min 30min after the FA injection. There was significant inhibition of the effect of FA-induced MSR activity. This inhibition was observed during the injection of haloperidol, and FA-induced neural activity decreased to predrug levels about 1hr after the haloperidol injection. This result was probably due to the effect of FA-induced MSR activity acting as an intermediary on the DA receptors.

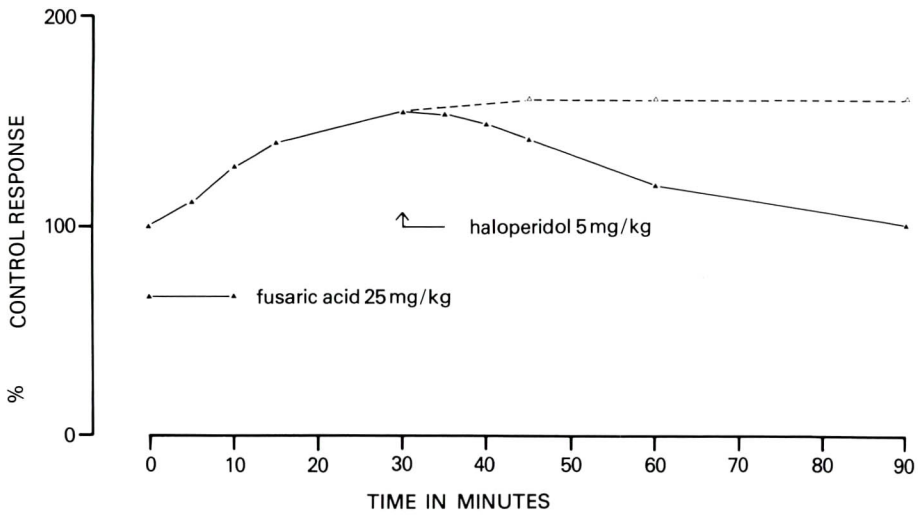

Fig. 2 Effects of FA followed by haloperidol on the monosynaptic reflex.

Each point is the average of three or four consecutive oscilloscope sweeps. Representative experiments 25mg/kg of FA and 5mg/kg of haloperidol are indicated by the line key in the lower left-hand corner and the length of the lines in the key indicates the time period during which each dose was infused.

△---△: 25mg/kg of FA + 5mg/kg of haloperidol

▲—▲: single dose of 25mg/kg of FA (without injection of haloperidol)

3) Effects of fusaric acid followed by L-dopa on the monosynaptic reflex. (Fig. 3)

The actions of 1—3, 4-dihydroxyphenylalanine (L-dopa) on spinal reflex activity have been considered to be due to the formation of excess NE rather than DA in the CNS (2, 5) and in the peripheral nervous system (18). Fig. 3 shows the results when L-dopa was administered at a dose of 2mg/kg/min 30min after the FA injection. FA-indcued neural activity increased by about 50% 30min after injection and then L-dopa was administered. The MSR neural activity was more potent after the L-dopa injection than

before. The effect of a single dose of dopa (20mg/kg) on the MSR was strongly potentiated within 5min after the start of the injection, and the percentage of increase observed was about 60% after 1hr (18). When FA and L-dopa were administered sequentially, L-dopa-induced neuronal activity markedly increased by about two-fold. In this study, the neural activity was facilitated by about 90%, 15 min after the L-dopa injection, and by about 150% 45min after the injection. This potentiation was about 2-fold greater than that with only L-dopa. The FA-induced MSR neural activity is due to the formation of excess DA.

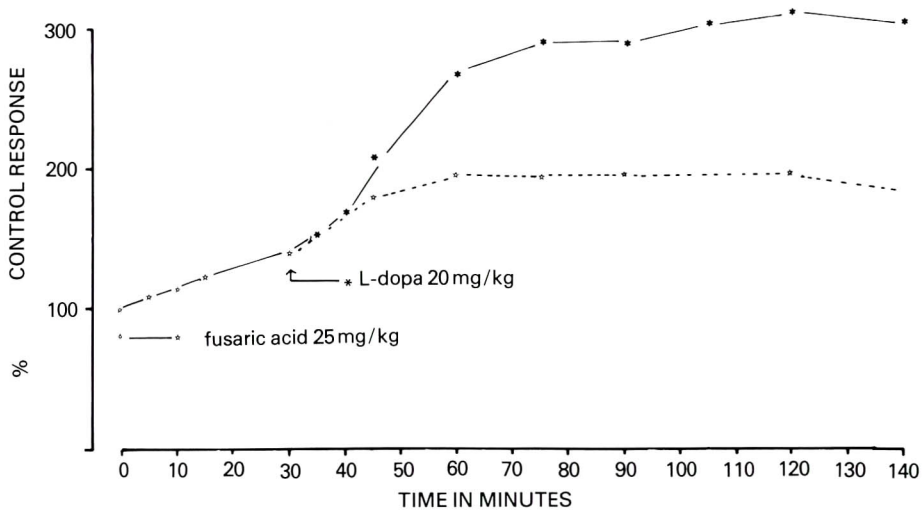

Fig. 3 Effects of FA followed by L-dopa on the monosynaptic reflex.
Each point is the average of three or four consecutive oscilloscope sweeps.
Infusion times of each drug are shown by the bar length.
★—★: 25 mg/kg of FA + 20 mg/kg of L-dopa
☆---☆: a single dose of 25 mg/kg of FA

4) Effects of FA followed by methysergide on the monosynaptic reflex. (Fig. 4)

An FA injection induced an increase of serotonin (5-HT) levels in the rat cerebellum (10). Therefore, we investigated the effects of a serotonergic receptor brocker on the FA-induced monosynaptic reflex. The injection of 0.5mg/kg of methysergide, a 5-HT receptor antagonist, did not affect the dopa-indcued monosynaptic neuronal activity, but completely antagonized the effect of 5-hydroxytryptophan, the precursor of 5-HT (6). In control animals this dose of methysergide invariably produced a depression of the monosynaptic spike of about 50%. Fig. 4 shows the effects of methysergide (0.2mg/kg) on the FA-induced monosynaptic reflex. When a dose of 25mg/kg of FA had increased the monosynaptic spinal reflex neural activity by about 60% of the preinjection level after 30min, methysergide was administered at a dose of 0.04mg/kg/min. The increased percentage of FA-induced potential was gradually depressed and this percentage was reduced to the pre-FA control level 50min after the methysergide injection.

In addition, the antagonistic action of methysergide was observed simultaneously with the methysergide injection, and this effect was similar to the inhibition of haloperidol (Fig. 2). Therefore, the increase of FA-induced MSR neural activity presumably caused to interact on the 5-HT and DA receptors.

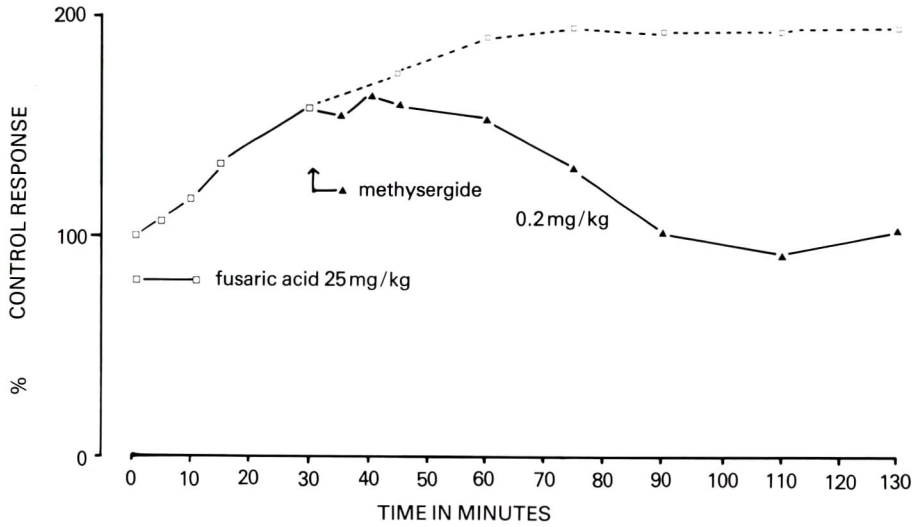

Fig. 4 Effects of FA followed by methysergide on the monosynaptic reflex.
Each point is the average of two or three consecutive oscilloscope sweeps. Representative experiments with a single dose of FA and combined doses of FA + methysergide are indicated by the line key. Infusion times of each drug are shown by the bar length.
▲—▲: 25 mg/kg of FA + 0.2 mg/kg of methysergide
□—□: a single dose of 25 mg/kg of FA

DISCUSSION

We previously reported that most hypertensive drugs which cause increases in levels of NE influence the stimulation of MSR neural activity (18). In the present investigation, we used an antihypertensive drug which is the most potent inhibitor of DBH to influence the MSR neural activity. This drug is 5-buthylpicolinic (fusaric) acid (FA), one of the picolinic acid derivatives. The administration of a single dose of 6 mg/kg of 5-buthylpicolinic acid calcium salt p.o. significantly reduced the blood pressure in normotensive and hypotensive subjects (16). The decrease in blood pressure was also demonstrated in SHR (spontaneously hypertensive rats) and normotensive rabbits after the i.v. administration of 50 mg/kg of FA and 5-(3′, 4′-dibromobutyl) picolinic acid (11). These results show that a more potent inhibitor of DBH markedly antagonizes hypertension together with the decrease of NE levels in the peripheral and central nervous system because FA can easily penetrate into the central nervous system even by p.o. administration route (14). The present study has shown that the administration of FA can increase the monosynaptic reflex neural activity in a dose-dependent manner (Fig. 1), and the onset of neural activity was significant-

ly potentiated by the administration of more than 10 mg/kg of FA. Therefore, we examined the pharmacological action of FA on the monosynaptic reflex. In the first place, we investigated whether or not there was an endogenous DA effect on FA induced monosynaptic neural activity. When FA administration was followed by haloperidol which did not affect the MSR at a single dose of 5 mg/kg or less, the FA-induced neural activity gradually began to decrease (Fig. 2). However, haloperidol blocked not only the DA receptor but also the α receptor, and the latter might not affect FA induced neural activity because FA does not accumulate as much NE in the spinal cord as DA (10). When L-dopa, a precursor of DA, was administered 30 min after FA injection, FA-induced neural activity was strongly facilitated, indicating an effect on the DA receptor sensitively (Fig. 3). Andén and Fuxe reported that the DBH inhibitor, bis (4-methyl-1-hemopiperazingl-thiocarbonyl) disulphide (FLA-63), blocked the formation of NE but not of DA by L-dopa (3). Therefore, it can be speculated that DA levels in the spinal cord were extensively prolonged by this sequential administration. This combined treatment-induced neural activity was greater than the L-dopa-induced neural activity. The single administration of 20 mg/kg of L-dopa caused an increase in neural potentiation of about 20% 15 min after injection, but this report showed that the neural potentiation increased about 60% above the control response. Furthermore, this combined administration hardly affected the blood pressure and the pulse rate for a while, but the blood pressure gradually rose 60 min after L-dopa treatment. These results showed that DA levels in the spinal cord mainly control the MSR neural activity, and FA-induced neural activity is probably modulated by DA. On the other hand, we reported that the treatment of cats with a DBH inhibitor prevented the facilitative effect of DA induced c-fiber reflex (13). This discrepancy makes it probable that the c-fiber reflex was mainly facilitated by NE but the monosynaptic reflex was significantly affected by DA, and the facilitation of c-fiber and MSR, respectively, was caused separately by different mechanisms. The mechanism of MSR neural activity appears to be more complex than that of the c-fiber reflex. Therefore, we studied what role another monoamine, i.e. 5-HT, plays in the neural activity of MSR. Hidaka reported that after three injections of FA, the concentration of cerebral 5-HT increased by 25% while that NE decreased by 40%. In addition, it has been shown that several DBH inhibitors also affect 5-HT metabolism. Moreover, Shibuya and Anderson proposed that 5-hydroxytryptophan (5-HTP) and tryptophan, the precursors of 5-HT, are facilitory and act on the spinal cord. In this report, it is noted that the 5-HT receptor blocker, methysergide, reduced FA-induced MSR neural activity (Fig. 4). This reduction was observed with the injection of 0.2 mg/kg of methysergide i.v. and the value slowly returned to control levels over a period of 1 hr. The single administration of 0.2 mg/kg of methysergide i.v., however, hardly induced any change in MSR neural activity. In Fig. 2, FA-induced neural activity was more rapidly antagonized by the injection of haloperidol, neuroleptics and a DA receptor blocker than by injection of a 5-HT receptor blocker. On the other side, Baker and Anderson reported that the single administration of methysergide can effectively antagonize the effect of

5-HTP on evoked potentials in the spinal cord but not that of L-dopa, while the single injection of haloperidol cannot inhibit the 5-HTP-induced neural activity but can inhibit that of L-dopa (6). Therefore, FA acts on different receptors and increases the MSR neural activity. These different receptors may be the DA receptor and the 5-HT receptor with the former playing a predominant role in FA-induced neural activity. Moreover, all of the DBH inhibitors must not decrease the blood pressure because changes in serum DBH activity are less sensitive as an index of sympathoadrenal activity than changes in blood pressure and heart rate (12). This is because not only some hypertensive drugs but also a few hypotensive drugs can be seemed to reflect the increase in MSR neural activity. However, the increase or decrease in blood pressure level can essentially induce almost no changes in the MSR neural activity of the spinal reflex.

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