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学位論文（要約）

Is milnacipran a promising agent to suppress impulsive behavior?

（ミルナシプランは有望な衝動性抑制薬であるか？）

２０１４年３月

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[Backgrounds]

Impulsive behavior can be viewed as everyday normal behavior. However, many studies have suggested that abnormally high levels of impulsivity is defined as one of core symptoms in attention-deficit hyperactivity disorder, borderline personality disorder, bipolar disorder, mania, and substance abuse and also defined as one of peripheral symptoms in schizophrenia, major depression, and anxiety disorder. Moreover, elevated impulsivity in mood disorder patients increases a risk of suicidal behavior. Nevertheless, only a few treatments, amphetamine, methylphenidate, and atomoxetine have been approved as therapeutic agents for suppressing elevated impulsivity. Therefore, developing novel therapeutic agents for disorders characterized by excessive levels of impulsivity are strongly needed.

I recently found that acute systemic administration of milnacipran, an antidepressant and a serotonin/noradrenaline reuptake inhibitor, could enhance impulse control in rats. However, the neural mechanisms underlying the effects of milnacipran on impulsive action remain unclear. Milnacipran increases not only extracellular serotonin and noradrenaline but also dopamine specifically in the ventromedial prefrontal cortex (vmPFC), which is one of brain regions responsible for impulsive action and is functionally comparable to the human prefrontal cortex, remains unknown.

It also remains to be examined whether milnacipran could suppress elevated impulsive behavior observed in animal models. A previous study demonstrated that selective excitotoxic lesion of the vmPFC impaired inhibitory control of impulsive action in rats.

[Objectives]

My goals were (1) to identify whether dopamine D_1-like and/or D_2-like receptors in the vmPFC mediates the milnacipran-enhanced impulse control in a 3-choice serial reaction time task (3-CSRTT) and (2) to investigate the effects of repeated milnacipran treatment on elevated impulsivity observed in rats with vmPFC-lesions and associated neural mechanisms.

[Method]

Rats' impulsive action was assessed by using a 3-CSRTT. When the task started, the house light was illuminated. After a fixed inter trial interval (ITI: 5 s), one of 3 holes was briefly illuminated (stimulus duration) in a random order so that a rat could not predict which hole would be illuminated. Nose poking during the ITI was recorded as a premature response, which is an index of impulsive action. Nose poking into the lit hole while it was illuminated or within 5 s of limited hold was recorded as a correct response, and the rat was rewarded by the delivery of a palatable food pellet. Nose poking into another hole was recorded as an incorrect response. When a rat failed to nose poke within the limited hold, it was recorded as an omission. After a food pellet had been delivered to and collected by the rat, the house light was switched off for 2 s to allow the rat to eat the pellet before the next trial was automatically started. The start of the next ITI was signaled by turning on the house light. Additional nose poking into any of the three holes prior to food collection was recorded as a perseverative response. Premature responses, incorrect responses, omissions, and perseverative responses resulted in a 5 s time out period during which the house light was extinguished. Because the trial was initiated automatically, I did not set a time restriction for this task. Each session consisted of 100 trials. Training was conducted for one session per day and 6 sessions per week. At the beginning of the training schedule, the stimulus duration lasted 30 s. Depending on individual performances, the stimulus duration progressively reduced to 1 s (15, 10, 5, 3, 2, 1.5, and 1 s). When a rat attained > 80% accuracy (the percentage of correct responses) and < 20 omissions in a session, the stimulus duration was reduced in the next session. I used six behavioral parameters described as follows:

(a) *Premature response* (no. per session)

(b) *Accuracy* (percentage of correct responses): [correct responses / (correct and incorrect responses)] ×100

(c) *Omission* (no. per session): [omission errors / total trials] ×100

(d) *Perseverative response* (no. per session)

(e) *Correct response latency* (s): the mean time between stimulus onset and

nose poke in the correct hole

(f) *Reward latency* (s): the mean time between reward delivery and nose poke in the food magazine

The completion of the training was determined as reaching the target phase (stimulus duration 1 s) and exhibiting stable performance. After completion of the training, the stimulus duration was fixed at 1 s regardless of performance. I set the criteria for determining stable performance as follows: the change in premature responses stayed within ± 25%, the accuracy stayed within ± 5%, and the number of omissions were less than 20 for at least 3 consecutive sessions.

For objective (1), after completing the training of the 3-CSRTT, rats were bilaterally injected with SCH23390, a selective D_1-like receptor antagonist, (0, 0.3, or 3 ng/side) or eticlopride, a selective D_2-like receptor antagonist, (0, 0.3, or 1 μg/side) into the vmPFC 50 minutes after acute intraperitoneal administration of milnacipran (0 or 10 mg/kg). Ten minutes after that, the 3-CSRTT was conducted.

For objective (2), selective lesions of the vmPFC were made using 0.09 M quinolinic acid in rats previously trained on the 3-CSRTT. Sham rats received phosphate buffered saline. Following a period of recovery, milnacipran (0 or 10 mg/kg/day × 14 days) was orally administered 60 min prior to testing on the 3-CSRTT. After 7 days of drug cessation, brains were removed and Western blotting, immunohistochemistry, electrophysiological analysis, and morphological analysis were conducted.

[Results and interpretations]

Systemic administration of 10 mg/kg of milnacipran decreased the number of premature responses. This milnacipran-induced decrease in the number of premature responses was blocked by injections of SCH23390, a selective D_1-like receptor antagonist, into the vmPFC, whereas intra-vmPFC injections of eticlopride, a selective D_2-like receptor antagonist, failed to inhibit the effect of milnacipran on impulsive action. In addition, intra-vmPFC SCH23390 injections without systemic administration of milnacipran caused no effect on impulsive

action. These results indicated that microinjections of 3 ng SCH23390 per side into the vmPFC elicited impulsive action by antagonizing the effects of milnacipran but not by antagonizing the effects of tonic endogenous dopamine.

vmPFC lesions provoked impulsive action without any changes in attentional, appetitive/motivational, and motor function. This lesion-induced increase in the number of premature responses was reversed by the repeated administration of milnacipran. Interestingly, the suppressive effect of repeated milnacipran on premature responses persisted for at least a week, even after cessation of the treatment. I then found that the repeated administration of milnacipran restored the protein levels of mature brain-derived neurotrophic factor (mBDNF) in the vmPFC of vmPFC-lesioned rats. Repeated milnacipran administration did not increase the number of neural cells but did recover the spine density and increased the proportion of mature spines in the vmPFC of vmPFC-lesioned rats. Furthermore, I found that repeated milnacipran treatment ameliorated impaired alpha-amino-3-hydroxy-5-methyl-4- isoxazole-propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) excitatory postsynaptic currents in the layer V pyramidal neurons in the vmPFC of vmPFC-lesioned rats. Finally, repeated milnacipran restored the decreased protein levels of PSD-95 but not Synapsin I in the vmPFC of vmPFC-lesioned rats. The findings suggested that repeated milnacipran treatment radically improved the dysregulation of impulsive action in vmPFC-lesioned rats, which could be attributable to the mBDNF-induced remediation of the spine density and the excitatory currents in the surviving layer V pyramidal neurons in the vmPFC.

[Conclusion]

My findings suggest that acute milnacipran could suppress impulsive behavior by enhancing D_1-like signaling in the prefrontal cortex. In addition, repeated milnacipran could suppress impulsive behavior on psychiatric patients even after the cessation of the treatment by upregulating the excitatory signaling in the impaired prefrontal cortex via stimulating BDNF pathway. “Is milnacipran a promising agent to suppress impulsive behavior?” The answer is “YES” at the

stage of animal study. I hope the proposed mechanisms underlying the suppressing effects of acute/repeated milnacipran on impulsive behavior will contribute to accelerating the development of anti-impulsive drugs.