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Block of the Tolerance to Ambulation Stimulant Effect of Scopolamine in Mice by Bethanecol, a Peripheral Cholinergic Drug

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Abstract: A behavioral tolerance to the ambulation-increasing effect of scopolamine (SCP: 2 mg/kg s.c.), a muscarinic anti-cholinergic drug, was induced following the repeated administration to mice at 3-day intervals. The combined administration of SCP + bethanechol (BET: 0.01, 0.03, 0.1 and 0.3 mg/kg s.c.), a peripheral muscarinic cholinergic drug, resulted in sensitization, although the single treatment with 0.03-0.1 mg/kg of BET did not modify the ambulation-increasing effect of SCP at the first administration. In addition, the treatment with BET (0.1 mg/kg) at post-SCP period of 5-20 min also induced the sensitization to SCP after the repeated administration. The post-SCP treatment with BET at 30 min and later produced the tolerance to SCP. The repeated administrations of BET alone did not change the sensitivity to the ambulation-increasing effect of SCP. Furthermore, the mice showing sensitization to SCP + BET, but not tolerance to SCP, demonstrated a cross-sensitization to methamphetamine (2 mg/kg s.c.). These results suggest that the tolerance to SCP is produced by the interaction of the stimulation of central dopaminergic system (reward effect) through the blockade of peripheral muscarinic cholinergic receptors (harmful effect), and that the latter effect overwhelms the former effect of SCP. It is also suggested that the selective inhibition of the peripheral anti-cholinergic effect of SCP increases the dependence liability of SCP, and psychotoxicity of anti-cholinergic drugs including SCP and psychomotor stimulant drugs such as methamphetamine.
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Key words: Scopolamine, Bethanechol, Methamphetamine, Behavioral tolerance, Central and peripheral cholinergic systems, Psychotoxicity, Mice.

Introduction

Scopolamine (SCP) has an antagonistic action on both the peripheral and central muscarinic acetylcholine receptors, and blocks the parasympathomimetic nervous system (Brunton et al., 2008). It has been considered that the blockade of central cholinergic systems (Mathura et al., 1997; Shannon and Peters, 2001; Chintoh et al., 2003), and the stimulation of central dopaminergic systems through the blockade of central muscarinic cholinergic receptors (Fink and Morgenstern, 1980) are involved in the SCP-induced hyperactivity.

SCP is self-administered by animals (Glick and Goido, 1982; Rasmussen and Fink-Jensen, 2000), and a short-term recreational use of SCP (Sussam and Ames, 2001) and Angel's trumpet (Greene et al., 1996) which is a plant containing muscarinic anti-choinergic drugs such as SCP and atropine has been reported, indicating dependence liability of SCP.

The repeated administrations of psychomotor stimulant drugs such as methamphetamine and cocaine (Kuribara and Hirabayashi, 1985; Kuribara, 1996, 2009) to mice induce sensitization to their ambulation-increasing effect. It has been considered that the processes and conditions of behavioral sensitization to psychomotor stimulant drugs are intimately related not only to the dependence liability of drugs (Piercem and Kumaresan, 2006) but also the psychotoxicity (Robinson and Becker, 1986; Tadokoro and Kuribara, 1986; Pert et al., 1990) induced by the repeated use of such drugs. Mesolimbic dopaminergic systems (Van der Heuval and Pasterkamp, 2008) play important roles in the behavioral and psycho-pharmacological activities,

including motivation (Matsumoto and Hikosaka, 2009), and drug dependence and abuse (Schultz, 2002; Piercem and Kumaresan 2006; Berridge, 2007; Ikemoto, 2007).

However, different from the characteristics of psychomotor stimulant drugs, it has been reported that the repeated administration of SCP to the mice at intervals of 1 day or longer resulted in a significant tolerance to the ambulation-increasing effect (Kuribara and Tadokoro, 1983, 1987), and that the tolerance was induced at an early post-SCP period (Kuribara, 2013) dependent on the environmental situations (Kuribara, 2011). The peripheral anti-cholinergic action of SCP causes harmful symptoms such as dry mouth and eyes (Bruston et al., 2008). It is therefore possible following the repeated administration of SCP that the peripheral harmful symptoms overwhelm the reward effect of SCP produced by the indirect stimulation of the central dopaminergic systems through blockade of the muscarinic cholinergic receptors, and that such interaction plays an important role in the induction of behavioral tolerance to the ambulation-increasing effect of SCP.

To confirm this possibility, the modification by bethanechol, a peripheral muscarinic acetylcholine receptor agonist, of the SCP-induced behavioral stimulation following the repeated administration was evaluated in mice. In addition, challenge administration of methamphetamine was carried out to the mice pretreated with the repeated administrations of SCP + BET for five times at interval of 3 days to assess the change in the sensitivity to the psychotoxicity of psychomotor stimulant drugs.

Materials and Methods

Animals

The experimental animals used were male mice of the ddY strain (SLA Japan, Hamamatsu). They were used at 6 weeks of age and a weight of 25-28 g. Groups of 10 mice each had been housed in Polycarbonate cages (20W X 25L X 15H cm) with free access to a solid diet (MF: Oriental Yeast, Tokyo) and tap water except during the behavioral tests. The breeding rooms were controlled to temperature; 23 ± 1 °C, relative humidity; 55 ± 3 %, and a 12:12-hr light-dark cycle; lights on between 06:00-18:00 hr. The temperature and relative humidity of experimental room were almost the same as those of the breeding room.

All the experimental treatments mentioned below were carried out according to "*The Guiding Principles for the Care and Use of Laboratory Animals*" of The Japanese Pharmacological Society.

Apparatus

The apparatus for measurement of the ambulatory activity of mice was a tilting-type "ambulometer" having 10 tilting-type Plexiglas activity cages of 20 cm in diameter and 15 cm in height (SMA-10: O'hara & Co., Tokyo). The apparatus could selectively detect the horizontal movement, but not turning, or vertical movements such as rearing, head movement or sniffing, of 10 mice.

Drugs

The drugs used were scopolamine hydrobromide (SCP: Sigma Chem., St. Louis, MO), bethanechol chloride (BET: Sigma Chem.) and methamphetamine hydrochloride (MA; Philopon: Dainippon-Sumitomo Pharm., Osaka). These drugs were dissolved in physiological saline, and subcutaneously (s.c.) administered at a constant volume of 0.1 ml/10 g body weight of the mouse independent of the doses of drugs. The doses of drugs were shown in the salt forms.

Experimental schedules

All the experimental treatments; the administration of SCP and BET, and measurement of ambulation of the mice, were carried out between 09:00-16:00 hr.

In the case of measurement of the activity of mice, they were individually put in the activity cages for 10 min, and then the administration of drugs was conducted. The activity of each mouse was measured at intervals for 90 and 180 min after administrations of SCP + BET and MA, respectively.

1) Repeated administration of SCP + BET and challenge administration of MA

Five groups of mice (10 mice each) were given SCP (2 mg/kg s.c.) + BET (0: saline, 0.01, 0.03, 0.1 and 0.3 mg/kg s.c.) for 5 times at intervals of 3 days. As the control administration, other 5 groups of 10 mice each were given BET (0, 0.01, 0.03, 0.1 and 0.1 mg/kg) alone. After each drug administration, the ambulatory activity of the mouse was measured for 90 min.

Three days after the final (5th) pretreatment with SCP (2 mg/kg) + BET (0, 0.01, 0.03, 0.1 and 0.3 mg/kg) or

BET (0, 0.01, 0.03, 0.1 and 0.3 mg/kg) alone, MA (2mg/kg s.c.) was challenge-administered to all groups of mice, and their ambulatory activities were measured for 180 min.

Other 10 mice of drug-naïve were administered MAP (2 mg/kg s.c.) as the control.

2) Repeated administration of SCP + post-SCP treatment with BET

Seven groups 10 mice each were administered SCP (2mg/kg s.c.) 5 times at intervals of 3 days. Each SCP administration was followed by the administration of BET (0.1 mg/kg s.c.) at 0 (simultaneous), 5, 10, 20, 30, 60 or 90 min (immediately after the end of measurement of ambulatory activity), and the ambulatory activities were measured for 90 min.

Statistical analysis

The mean activity counts in each group were analyzed by one-way analysis of variance (ANOVA). Post-hoc analyses were carried out by Bonferroni test. Values of p less than 0.05 were considered significant.

Results

Repeated administration of SCP + BET and challenge administration of MA

Table 1 shows the mean overall activity counts of mice following repeated co-administration of SCP (2 mg/kg) + BET (0, 0.01, 0.03, 0.1 and 0.3 mg/kg) at 3-day intervals.

Although there was no significant difference in the activity counts at the first administration, SCP (2 mg/kg) alone induced progressive decrease in the ambulation-increasing effect, SCP + BET (0.01 mg/kg) tended to increase the effect, and SCP + BET (0.03 and 0.1 mg/kg) induced a significant sensitization following the repeated administrations.

The mice given SCP + BET (0.3 mg/kg) showed significantly lower activity count at the first administration, and no significant change in the activity count was demonstrated following the repeated administration.

The activity counts of the mice treated with BET alone were very low (10-39 counts) at the first administration, and no significant change in the activity counts was demonstrated throughout the five repeated administrations (data not shown). No significant change in the sensitivity to SCP was observed in the mice pretreated with BET (data not shown).

Table 2 shows mean 180 min activity counts following the challenge administration of MA to the groups of mice that had been pretreated with BET alone or SCP + BET.

The groups of mice pretreated with SCP or BET alone did not show significant change in the sensitivity to the challenge-administered MA. In contrast, the groups of mice pretreated with SCP + BET showed significantly higher sensitivity to MA.

Repeated administration of SCP + post-SCP treatment with BET

As shown in Table 3, the activity counts at the first administration of SCP + post-SCP treatment with BET were almost the same among the groups of mice regardless of the doses of BET. However, the repeated administration of SCP + post-SCP treatment with BET induced sensitization when BET treatment was carried out at 0, 5, 10 and 20 min, and tolerance when BET treatment was carried out at 30, 60 or 90 min.

Table 1. Mean activity counts of mice following the repeated co-administration of scopolamine (SCP: 2 mg/kg s.c.) + bethanechol (BET: 0, 0.01, 0.03, 0.1 and 0.3 mg/kg s.c.) for 5 times at 3-day intervals.

Doses of drugs	1st	2nd	3rd	4th	5th
SCP alone	523±61	262±28*	172±15*	124±15*	106±13*
SCP + BET (0.01)	557±71	510±67	565±55	455±86	416±67
SCP + BET (0.03)	572±67	668±71	785±89$	793±80$	817±92$
SCP + BET (0.1)	530±55	628±80	722±99$	740±83$	769±87$
SCP + BET (0.3)	312±57	363±49	389±47	413±59	431±61

N=10 in each group. *: significantly lower ($p<0.05$) vs. the value at the 1st administration within group.
$: significantly higher ($p<0.05$) vs. the value at the 1st administration within each group.

Table 2. Mean activity counts after the challenge-administration of methamphetamine (2mg/kg s.c.) to the mice pretreated with scopolamine (2 mg/kg s.c.) + bethanechol (0, 0.01, 0.03, 0.1 and 0.3 mg/kg s.c.) or bethanechol alone for 5 times at 3-day intervals.

Pretreatments		Activity counts following methamphetamine
Saline		1756 ± 219
Bethanechol	0.01 mg/kg	1692 ± 241
	0.03	1716 ± 229
	0.1	1670 ± 264
	0.3	1688 ± 225
Scopolamine alone		1994 ± 265
Scopolamine + Bethanechol 0.01 mg/kg		2356 ± 309
Scopolamine + Bethanechol 0.03		2911 ± 391*,$
Scopolamine + Bethanechol 0.1		3308 ± 446*,$
Scopolamine + Bethanechol 0.3		2503 ± 338
No treatment		1701 ± 247

N=10 in each group. *: $p<0.05$ vs. the value of saline-pretreated group. $: $p<0.05$ vs. the value of mice pretreated with scopolamine alone.

Table 3. Mean activity counts of mice following the repeated administration of scopolamine (SCP: 2 mg/kg s.c.) + post-SCP treatment with bethanechol (BET: 0.1 mg/kg s.c.) for 5 times at 3-day intervals.

Doses of drugs	1st	2nd	3rd	4th	5th
SCP→(0min)→BET	550±76	689±81	759±98*	807±91*	837±99*
SCP→(5min)→BET	523±62	568±57	658±68	739±77*	840±85*
SCP→(10min)→BET	543±76	568±71	608±87	685±83	783±77*
SCP→(20min)→BET	552±67	628±52	660±70	643±93	715±72*
SCP→(30min)→BET	559±72	339±53$	302±55$	270±33$	212±35$
SCP→(60min)→BET	591±85	213±42$	137±22$	141±30$	133±24$
SCP→(90min)→BET	572±67	228±35$	108±19$	110±13$	105±12$

N=10 in each group. *: significantly higher ($p<0.05$) vs. the value at the 1st administration within group. $: significantly lower ($p<0.05$) vs. the value at the 1st administration within each group.

Gross observation

The mice given BET alone and combination of SCP + BET (0.3 mg/kg) showed parasympathomimetic symptoms such as increase in saliva and tear.

Discussion

In consistent with the previous reports (Kuribara, 2011; Kuribara and Tadokoro, 1983, 1987), the repeated administration of SCP alone to the mice at intervals of 3 days induced tolerance to the ambulation-increasing effect. It has been considered that such tolerance to SCP is highly dependent on the environment, and that the peripheral anti-muscarinic symptoms play an important role in the induction of tolerance to SCP (Kuribara, 2011). The central dopaminergic system (Fink and Morgenstern, 1980) and cholinergic systems (Mathura et al., 1997; Shannon and Peters, 2001; Chintoh et al., 2003) are involved in the SCP-induced hyperactivity. Generally, the repeated administrations of psychomotor stimulants

and narcotic analgesics to the mice induce significant sensitization to their ambulation-increasing effects through stimulation of the mesolimbic dopaminergic systems (Kalivas and Stewart, 1991; Kuribara, 1995). The author reported that the induction of tolerance to the ambulation-increasing effect of SCP was highly dependent on the interaction of the anti-cholinergic effect and the movement in the activity cage at an early post-SCP period (Kuribara, 2011, 2013).

Following to these pharmacological and behavioral evidences, it is supposed that the repeated administrations of SCP induce sensitization to its ambulation-increasing effect when the peripheral, but not central, muscarinic anti-cholinergic effect is selectively inhibited at an early post-SCP period. To confirm this hypothesis, in this study, BET was used to selectively block the peripheral anti-cholinergic effect of SCP.

Although BET alone did not change the sensitivity to SCP, the mice treated with SCP + BET (0.03 and 0.1 mg/kg) produced not only significant enhancement of the ambulation-increasing effect, but also cross sensitization to MAP. In is also notable that the blockade of SCP tolerance by BET was effective when BET treatment was carried out at post-SCP period of 0-20 min. This result is consistent with the previous report (Kuribara, 2011, 2013) that the induction of SCP tolerance could be attenuated by the physical restriction of ambulation or blockade of the anti-cholinergic effect of SCP during the early post-SCP period, suggesting that the combined experience of ambulation with the peripheral cholinergic symptoms at the early post-SCP period is important to induce the tolerance to SCP.

These findings strongly suggest that, although SCP stimulates central dopaminergic systems through the blockade of the muscarinic cholinergic receptors which may be related to the reward effect of SCP (Glick and Goido, 1982; Rasmussen and Fink-Jensen, 2000), the harmful symptoms caused by peripheral muscarinic anti-cholinergic effect of SCP is much stronger than the reward effect.

The behavioral sensitization to psychomotor stimulants is considered to be intimately related to the reward effects, i.e., dependence liability (Piercem and Kumaresan 2006; Berridge, 2007; Ikemoto, 2007) and psychotoxicity (Robinson and Becker, 1986; Tadokoro and Kuribara, 1986; Pert et al., 1990).

It is therefore suggested from the present results that, although severe dependence and abuse are rare for the muscarinic anti-cholinergic drugs (Sussam and Ames, 2001) and Angel's trumpet (Greene et al., 1996; Brunton et al., 2008), which contains muscarinic anti-cholinergic drugs such as SCP and atropine, blockade of the peripheral muscarinic cholinergic effect by the drugs such as bethanechol may enhance the dependence liability of anti-cholinergic drugs and the psychotoxicity of both anti-cholinergic drugs and psychomotor stimulants.

Conclusion

The induction of tolerance to the ambulation-increasing effect of SCP (2 mg/kg s.c.), an antagonist of muscarinic cholinergic receptors, was blocked, but rather induced sensitization when bethanechol (0.03 and 0.1 mg/kg s.c.), a peripheral muscarinic cholinergic drug, was treated at early post-SCP period of 0-20 min. The repeated administrations of SCP + BET (0.03 and 0.1 mg/kg) resulted in the cross sensitization to methamphetamine (2 mg/kg s.c.). These results suggest that the tolerance to the ambulation-increasing effect of SCP is induced by the experience of the harmful symptoms caused by the peripheral muscarinic cholinergic effect at the early post-SCP period. It is also considered that the combined abuse of anti-muscarinic cholinergic drug with peripheral muscarinic drugs may increase the risks of abuse and psychotoxicity of not only anti-muscarinic cholinergic drugs but also psychomotor stimulants.

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末梢性ムスカリン型コリン作動薬bethanecholによるScopolamineのマウス移所運動促進作用に対する耐性形成の阻止

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抄録： Scopolamine（SCP: 2 mg/kg s.c.）を3日間隔で反復投与すると、マウスの移所運動促進作用に対する耐性形成が形成される。SCP（ムスカリン型抗コリン薬）と末梢ムスカリン型コリン作動薬のbethanechol（BET: 0.01, 0.03, 0.1, 0.3 mg/kg s.c.）を併用すると、初回投与時では、BETは高用量（0.3 mg/kg）を除いて、SCPの移所運動促進作用に影響しなかったが、併用投与を反復すると増感現象が引き起こされた。SCP耐性の阻止および増感現象は、SCP投与から20分以内にBETを処置した場合にのみ認められ、30分以降の処置では耐性が引き起こされた。BETおよびSCPの単独投与を反復経験したマウスは覚せい剤methamphetamine（2 mg/kg s.c.）に対して感受性変化を示さなかったが、SCP ＋ BET（00.03, 0.1 mg/kg）を反復経験したマウスは、覚せい剤に対して交差増感を示した。これらの結果は、抗コリン薬を投与した場合、中枢のムスカリン型アセチルコリン受容体（MAchR）の阻害は報酬的効果（依存性）を現すが、同時に末梢のMAchRを介する嫌悪作用によってブロックされること、また、末梢のMAchRを遮断しながら抗コリン薬を反復乱用すると、抗コリン薬に対してのみならず、覚せい剤のような精神刺激薬の精神毒性が増強される可能性も示唆している。
（別刷請求先：栗原　久）

キーワード： Scopolamine, Bethanechol、Methamphetamine、行動耐性、中枢性と末梢性アセチルコリン神経系、精神毒性、マウス