ences were observed in the conjugation rate of magnolol between the groups.

Discussion

In a previous study, we identified three major components in the urine of patients receiving Saiboku-To treatment (Homma et al 1992) as magnolol, dihydroxydihydro-magnolol, and liquiritigenin. We expected these components to play an important role for corticosteroid-sparing effects on asthmatic patients. Our particular interest was focused on magnolol because this component showed inhibitory activity against 11β-hydroxysteroid dehydrogenase (unpublished data).

We improved our original extraction procedure and added an HPLC stage for quantitation of magnolol in urine. Thus, we were able to determine magnolol with an improved detection limit of 2·5 ng. Sensitivity and specificity of this method are comparable with those of LC-MS methods using radiolabelled magnolol (Hattori et al 1986). We applied our method to determination of the free and total magnolol after a single administration of Saiboku-To as shown in Table 2.

A single dose of 5 g Saiboku-To contained 2·1 mg magnolol. Around 10% of the dosed magnolol was excreted in the urine collected for 9 h following administration. This recovery rate was comparable with that obtained in animal experiments (Hattori et al 1986). Individual variations of magnolol excretions were observed at the same single dose of Saiboku-To per subject, which could not be explained by body weight differences. Extended studies employing our method and patient groups such as responders and non-responders under long-term Saiboku-To treatment will be useful in determining the clinical implications of magnolol.

This work was supported by the Ministry of Education in Japan (Grant in Aid No. 03857345). We are grateful to Professor Y. Sashida for excellent discussions and to Mr T. Yamada and Ms C. Higuchi for their technical assistance.

References

Fujita, M., Itokawa, H., Sashida, Y. (1973a) Studies on the components of *Magnolia obovata* thunb. II. On the components of methanol extract of the bark. Yakugaku Zasshi 93: 424–428 (in Japanese)

Fujita, M., Itokawa, H., Sashida, Y. (1973b) Studies on the components of *Magnolia obovata* thunb. III. Occurrence of magnolol and honokiol in *M. obovata* and other allied plants. Yakugaku Zasshi 93: 429–434 (in Japanese)

Hattori, M., Endo, Y., Takebe, S., Kobayashi, K., Fukasaku, N., Namba, T. (1986) Metabolism of magnolol from magnoliae cortex. II. Absorption, metabolism and excretion of [ring-^{14}C] magnolol in rats. Chem. Pharm. Bull. 34: 158–167

Homma, M., Hirano, T., Oka, K. (1991) pH-dependent column fractionation for characterization of endogenous digoxin-like immunoreactive factors in pregnant urine. Biomed. Chromatogr. 5: 175–179

Homma, M., Oka, K., Yamada, T., Niitsuma, T., Itoh, H., Takahashi, N. (1992) A strategy for discovering biologically active compounds with high probability in traditional Chinese herb remedies: an application of Saiboku-To in bronchial asthma. Anal. Biochem. 202: 179–187

Nagano, H., Kobayashi, S., Nakajima, S., Egashira, Y. (1988) Long-term clinical evaluation of Saiboku-To, an anti-asthmatic agent, in the treatment of bronchial asthma (multicenter open trial). Respiration Research 7: 76–87 (in Japanese)

Oka, K., Ohki, N., Noguchi, M., Matsuoka, Y., Irimajiri, S., Abe, M., Takizawa, T. (1984) Extraction monitoring and rapid flow fractionation for determination of serum corticosteroids. Anal. Chem. 56: 2614–2617

J. Pharm. Pharmacol. 1993, 45: 841–843
Communicated October 16, 1992

DAU 6215, a novel 5-HT$_3$-receptor antagonist, selectively antagonizes scopolamine-induced deficit in a passive-avoidance task, but not scopolamine-induced hypermotility in rats

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Abstract—This study examined the effects of DAU 6215, a selective 5-HT$_3$-receptor antagonist, on either impairment of a passive-avoidance task or hypermotility, both caused by scopolamine in rats. In the first experiment, scopolamine (0·75 mg kg^{-1}, i.p.) disrupted acquisition of a one-trial 'step through' passive-avoidance response. Pretreatment with DAU 6215 (1, 10, 30 and 100 μg kg^{-1}, i.p.) antagonized this deficit induced by scopolamine, with a bell-shaped dose-response curve. Scopolamine (0·75 mg kg^{-1}, i.p.) produced a significant increase in locomotor activity which was unaffected by pretreatment with DAU 6215 (10 and 30 μg kg^{-1}, i.p.). The present results further support the suggestion that 5-HT$_3$-receptor antagonists may prevent the memory disturbance caused by a reduction in central cholinergic function in the rat. The inefficacy shown by DAU 6215 on hyperactivity induced by scopolamine appears to rule out the possibility of a pharmacokinetic interference between DAU 6215 and scopolamine.

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There is ample evidence indicating that 5-hydroxytryptamine (5-HT) mechanisms play a significant role in learning and memory processes (McEntee & Crook 1991). Stimulation of the 5-HT-ergic nervous system has a negative influence on learning and memory (Fibiger et al 1978), while 5-HT-ergic antagonists produce an enhancement of cognitive functions (Altman & Normile 1986).

Among the compounds acting on the different 5-HT-receptor subtypes, 5-HT$_3$-receptor antagonists have been reported to enhance learning and memory performance in animals (Barnes et al 1990; Chugh et al 1991a, b).

Results from these studies demonstrated that 5-HT$_3$-receptor antagonists inhibited the impairment in performance caused by cholinergic deficits in a passive avoidance task in mice (Chugh et al 1991a) and in a working memory task in rats (Barnes et al 1990).

The validation of laboratory tests, or animal models, designed to investigate cognition enhancing drugs is far from established

(Heise 1987). No clearly effective reference compounds have as yet been discovered. As a consequence, the use of a variety of versions of, for example, the passive-avoidance procedure by different laboratories might lead to contradictory results (Sanger & Joly 1990).

With these general considerations concerning animal models in mind, we decided to investigate the potential anti-amnestic effects of DAU 6215 ((3-α-tropanyl)1H-benzimidazolone-3-carboxamide), a novel 5-HT_3-receptor antagonist (Giraldo et al 1989), on scopolamine-induced impairment of passive-avoidance response in rats, an animal model not yet investigated with 5-HT_3-receptor antagonists.

A possible pharmacokinetic interaction between DAU 6215 and the cholinergic system was evaluated by testing the effect of DAU 6215 on scopolamine-induced hyperactivity in rats.

Materials and methods

Animals. Male Sprague-Dawley rats (Charles River, Calco, Italy), 175–200 g, were kept in a regulated environment (21 ± 1°C, 50–55% r.h. on a 12 h light-dark cycle, lights on at 0700 h) and maintained on standard pellet diet (Mucedola s.r.l., Milano, Italy).

Passive avoidance. Apparatus. The passive-avoidance apparatus was composed of a dark compartment (30 × 30 × 30 cm) with a grid floor and a compartment (30 × 30 × 30 cm) illuminated from above, the two compartments being separated by a vertically sliding door (10 × 8 cm). The light intensity at the floor of the lit compartment was 350 lx. Electric shocks were delivered to the grid floor by an isolated stimulator.

Procedure. Rats were divided into groups of 10 animals. For the training (acquisition) session, each rat was placed gently in the light compartment and 10 s later the door was opened. As soon as the rat had moved into the dark compartment and the door had been shut, a 1·6 mA foot-shock was applied for 1 s. The rat was immediately removed from the apparatus and returned to the home cage.

For the test (retention) session, after 24 h each rat was placed into the light compartment again and the step-through latency was recorded. The test was stopped when the rat entered the dark compartment or when it failed to do so in less than 180 s. In order to produce an impairment of the avoidance task, the animals were treated intraperitoneally with 0·75 mg kg^{-1} scopolamine 30 min before acquisition, i.e. at a dose required to induce a submaximal impairment of acquisition, in our experimental conditions. The control group received the vehicle alone (0·9% NaCl). DAU 6215 (1, 10, 30 and 100 μg kg^{-1}) or vehicle (0·9% NaCl) was given intraperitoneally 15 min before scopolamine. The retention test was performed 24 h later by an observer unaware of the treatment given.

Locomotor activity. Apparatus. The apparatus consisted of four 40 × 40 × 30 cm plexiglass boxes housed within an outer chamber equipped with 16 photocells positioned 4 cm above the grid floor. The number of interruptions (i.e. activity count) by each animal of the photoelectric beams was recorded by a computer system (Activity Monitor mod. 540, Giunta Scientific Instruments).

Procedure. The rats were injected intraperitoneally with DAU 6215 at doses of 10 and 30 μg kg^{-1} or with the vehicle. After 15 min the rats received an intraperitoneal injection of 0·75 mg kg^{-1} scopolamine (i.e. a dose eliciting a submaximal hyperlocomotion) and 30 min later their locomotor activity was recorded for 5 min, after 3 min habituation to the apparatus. Rats of the control groups received the vehicle alone (0·9% NaCl). Eight animals for each group were used.

Drugs. Scopolamine HBr was obtained from Sigma (St Louis, MO). DAU 6215 Cl was from Boehringer Ingelheim, Italy. The compounds were dissolved in 0·9% NaCl (saline) and administered in a volume of 5 mL kg^{-1}. Doses of compounds were calculated as free base.

Statistics. Data concerning locomotor activity were expressed as mean activity counts ± s.e. Comparison amongst groups was made by two-way analysis of variance followed by Tukey's test. The experimental data of the passive avoidance test were expressed as median retention latency with interquartile ranges and were evaluated statistically by means of a Mann–Whitney U-test comparing all treatments, including controls, with the scopolamine-treated group.

Results

Passive avoidance. Non-shocked rats usually found the entrance into the dark compartment within a few seconds and entered it without hesitation, both during training (about 20 s) and during the retention test (about 15 s) (data not shown). Rats of the control groups, i.e. those receiving electric shock, showed prolonged latency to re-enter the dark compartment and all of them remained in the light compartment for more than 180 s (Fig. 1).

Scopolamine, 0·75 mg kg^{-1} given intraperitoneally 30 min before training, significantly reduced the step-through latency in the retention test. Pretreatment with DAU 6215 attenuated the deficit induced by scopolamine with a bell-shaped dose-response curve, i.e. groups treated with 10 or 30 μg kg^{-1} were significantly improved compared with scopolamine groups ($P < 0·01$ and $P < 0·05$, respectively); the group treated with 1 μg kg^{-1} was not significantly impaired in comparison with the control gorup, but neither was it significantly improved compared with the scopolamine group; finally, the group treated with 100 μg kg^{-1} was significantly impaired in comparison with the control group

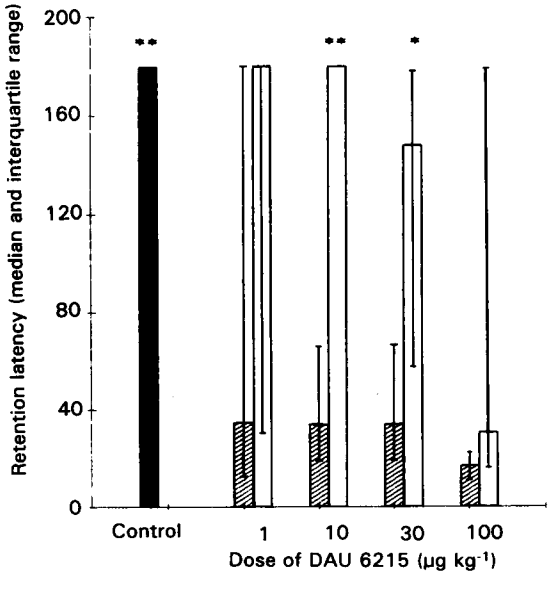

FIG. 1. Effects of DAU 6215 on scopolamine-induced impairment of passive-avoidance learning in rats. DAU 6215 and scopolamine (0·75 mg kg^{-1}) were injected intraperitoneally 45 min and 30 min before the acquisition, respectively. The retention test was run 24 h after the acquisition. Ten animals for each dose and scopolamine groups were used. Controls (n = 30) were pooled. ■, Control; ■, scopolamine; □ DAU 6215 + scopolamine. ** $P < 0·01$, * $P < 0·05$ in comparison with scopolamine groups.

Table 1. Effect of DAU 6215 on hyperactivity caused by scopolamine in rats.

Treatment	Activity counts in 5 min	
	Vehicle	Scopolamine
Control	32·7±4·5	57·2±6·2**
DAU 6215 10 μg kg^{-1}	28·6±2·3	56·4±4·2
Control	26·6±4·2	63·4±8·6**
DAU 6215 30 μg kg^{-1}	25·6±2·2	46·5±9·1

Data represent mean±s.e. from 8 rats. DAU 6215 was administered 15 min before scopolamine (0·75 mg kg^{-1}). Compounds were injected intraperitoneally. Locomotor activity was recorded 30 min after scopolamine. ** $P<0·01$ vs control groups.

($P<0·05$) (Fig. 1). None of the doses of DAU 6215 tested modified latency during the training session.

Locomotor activity. Intraperitoneal administration of 0·75 mg kg^{-1} scopolamine 30 min after injection, caused a significant increase in rat locomotor activity relative to vehicle alone (Table 1).

Intraperitoneal administration of DAU 6215 (10 or 30 μg kg^{-1}) was without significant effects on spontaneous locomotor activity.

Pretreatment with DAU 6215 at both doses did not significantly reduce the hyperactivity caused by scopolamine in rats (Table 1).

Discussion

DAU 6215, a selective 5-HT$_3$-receptor antagonist, like other chemically different 5-HT$_3$-receptor antagonists, was able to antagonize the amnestic effects of the antimuscarinic, scopolamine (Izquierdo 1989) in a passive-avoidance procedure in rats, a learning and memory paradigm not yet investigated with 5-HT$_3$-receptor antagonists in this animal species.

DAU 6215 did not antagonize the hyperactivity caused by scopolamine, a behavioural effect produced by antimuscarinic drugs which does not involve cognitive performance (Bushnel 1987). The failure of DAU 6215 in antagonizing scopolamine-induced hypermotility indicates, therefore, that the antagonism towards scopolamine in the passive avoidance-paradigm does not depend on a pharmacokinetic interaction.

It has been demonstrated that the 5-HT$_3$ receptor is present in the brain (Peroutka 1990) and that its activation may mediate inhibition of acetylcholine release (Barnes et al 1989). In animal studies, drugs which increase acetylcholine levels antagonize the memory deficits produced by scopolamine (Bartus 1978), suggesting that blockade of central 5-HT$_3$ receptors by DAU 6215 may counteract the memory deficit caused by scopolamine through a cholinergic mechanism. However, it has also been reported that cholineresterase inhibitors, which would be expected to enhance the effects of acetylcholine at cholinergic receptors, attenuated scopolamine-induced hyperactivity (Shannon & Peters 1990).

Taken together, these findings suggest that increase in locomotor activity and cognitive impairment, both caused by scopolamine in rats, are mediated by neural pathways differentially affected by 5-HT$_3$-receptor antagonists.

In conclusion, this study provides further evidence that 5-HT$_3$-receptor antagonists were able to restore memory deficits caused by cholinergic hypofunction in different paradigms in animals. Moreover, the interactions between 5-HT$_3$ mechanism and the cholinergic system occurs mainly when learning and memory functions are involved.

References

Altman, H. G., Normile, H. J. (1986) Enhancement of memory of a previously learned aversive habit following pre-test administration of a variety of serotonergic antagonists in mice. Psychopharmacology 90: 24–28

Barnes, J. M., Barnes, N. M., Costall, B., Naylor, R. J., Tyers, M. B. (1989) 5-HT$_3$ receptors mediate inhibition of acetylcholine release in cortical tissue. Nature 338: 762–763

Barnes, J. M., Costall, B., Coughlan, J., Domeney, A. M., Gerrard, A., Kelly, M. E., Naylor, R. J., Onaivi, E. S., Tomkins, D. M., Tyers, M. B. (1990) The effects of ondansetron, a 5-HT$_3$ receptor antagonist, on cognition in rodents and primates. Pharmacol. Biochem. Behav. 35: 955–962

Bartus, R. T. (1978) Evidence for a direct cholinergic involvement in the scopolamine-induced amnesia in monkeys. Pharmacol. Biochem. Behav. 9: 833–836

Bushnel, P. J. (1987) Effects of scopolamine on locomotor activity and metabolic rate in mice. Pharmacol. Biochem. Behav. 26: 195–198

Chugh, Y., Saha, N., Sankaranarayanan, A., Datta, H. (1991a) Enhancement of memory retrieval and attenuation of scopolamine-induced amnesia following administration of 5-HT$_3$ antagonist ICS 205–930. Pharmacol. Toxicol. 69: 105–106

Chugh, Y., Saha, N., Sankaranarayanan, A., Sharma, P. L. (1991b) Memory enhancing effects of granisetron (BRL 43694) in a passive avoidance task. Eur. J. Pharmacol. 203: 121–123

Fibiger, H. C., Lepiane, F. S., Philips, A. G. (1978) Disruption of memory produced by stimulation of the dorsal raphe nucleus: mediation by serotonin. Brain Res. 155: 380–386

Giraldo, E., Monti, L., Turconi, M., Nicola, M., Donetti, A., Ladinsky, H. (1989) Binding affinities of a novel class of serotonin 5-HT$_3$ receptor antagonists. Serotonin: from Cell Biology to Pharmacology and Therapeutics, Abstr., Florence, Italy

Heise, G. A. (1987) Facilitation of memory and cognition by drugs. Trends Pharmacol. Sci. 8: 65–68

Izquierdo, I. (1989) Mechanism of action of scopolamine as an amnestic. Trends Pharmacol. Sci. 10: 175–177

McEntee, W. J., Crook, T. H. (1991) Serotonin, memory and the ageing brain. Psychopharmacology 103: 143–149

Peroutka, S. J. (1990) Receptor families for 5-hydroxytryptamine. J. Cardiovasc. Pharmacol. 16: S8–S14

Sanger, D. J., Joly, D. (1990) Psychopharmacological strategies in the search for cognition enhancers. Pharmacopsychiatry 23: 70–74

Shannon, H. E., Peters, S. C. (1990) A comparison of the effects of cholinergic and dopaminergic agents on scopolamine-induced hyperactivity in mice. J. Pharmacol. Exp. Ther. 255: 549–553