Possible Mechanism Involved in the Sedative Activity of Jujubasaponins I in Mice

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Received 7 January 2013; revision 16 January

2013; accepted 16 January 2013.

doi: 10.1111/cns.12070

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Insomnia is a most common health complaint in the clinical practice. Approximately 30% of the general population in the world experiences some insomnia-related symptoms occasionally, and 10% of the population suffers from chronic and persistent insomnia [1]. Several lines of evidence have shown that many consequences attribute to insomnia, such as fatigue, stress, and memory deficits [2]. Therefore, it is urgent for those basic and clinical pharmacologists to develop the effective pharmacotherapy to treat insomnia [3].

Suanzaoren Decoction, which was recorded originally as compound traditional Chinese medicine decoction in the synopsis of the golden chamber, has been used widely to treat insomnia in folks for 2000 years in China. However, there has been no report on the chemical compounds related to sedative activity from the preparation so far. In the previous experiment, we isolated the large quantity of jujubasaponins I (Juju I) (Figure 1A) from this decoction by the method of bioactivity guide and found it the significant sedative activity which had not been reported yet. Here, we further explored the possible sedative mechanism of this compound to develop it more reasonable.

Each experimental group consisted of 20 ICR mice (18–22 g, equal numbers of male and female). They were housed at $21 \pm 1^\circ\text{C}$ under a 12 h light/12 h dark cycle (light on at 7:00 am) and had free access to standard pellet diet (Purina chow) and tap water. Each animal was used once in these experiments. The experimental protocols were approved by the Animal Care and Use Commit-

tee of our institute. The experiments were performed from 13:00 to 16:30 in a quiet room, which maintained the same condition mentioned previously.

To examine the actual hypnosis of Juju I, indexes of hypnosis were recorded in the pentobarbital sodium-induced sleep time test [4]. These indexes were recorded as follows: (1) those animals that stopped moving about in the box within 5 min after pentobarbital injection were immediately transferred to another box, where those individuals that stayed immobile for more than 1 min when positioned delicately on its back were judged to be asleep. The time that elapsed from receiving pentobarbital to each animal losing its righting reflex represented the latency to onset of sleep (T_1), (2) the animals were observed constantly, and the period from sleep to awakening (characterized by righting spontaneously of the animals and not losing righting reflex within 1 min when positioned delicately on its back) was noted (T_2), (3) the period from recovery of the righting reflex to these mice beginning to move freely was recorded as movement convalescence time (T_3), which could reflect the duration of effect of the drug on CNS after waking up.

Mice were injected intraperitoneally with Juju I (5, 10, 20, 40, and 80 mg/kg) or the vehicle (10 mL/kg), respectively, 15 min before injection of pentobarbital sodium (45 mg/kg, i.p.). Diazepam (1 mg/kg, i.p.) was injected as positive control 20 min before the test. From these results, Juju I, at the dose of 20, 40, and 80 mg/kg, could not affect the recovery of the ability to move

Figure 1 (A) The chemical structure of Jujul. (B) Mice were injected intraperitoneally with Jujul (5, 10, 20, 40 and 80 mg/kg) or the vehicle (10 mL/kg), respectively, 15 min before injection of pentobarbital sodium (45 mg/kg, i.p.). Diazepam (1 mg/kg, i.p.) was injected as positive control 20 min before the test. T1: sleep latency, T2: sleeping time, T3: movement convalescence time. Each group represents the mean $\pm$ SEM ($n = 20$). Asterisks indicated significant difference from control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (ANOVA followed by Dunnett's test). (C) 15 min or 25 min before pentobarbital sodium was injected, mice were treated with Jujul (20 mg/kg, i.p.), the vehicle (10 mL/kg, i.p.) or prazosin (2 mg/kg, i.p.), respectively. In combination, mice were treated with Jujul 10 min after prazosin or NA (0.2 mg/kg, s.c.) was administered intraperitoneally. 15 min later, pentobarbital sodium was injected. T1, T2 and T3 were observed. In addition, mice treated with prazosin (2 mg/kg, i.p.) were also administered intraperitoneally with NA in advance, and T1, T2, T3 were observed. T1: sleep latency, T2: sleeping time, T3: movement convalescence time. Each group represents the mean $\pm$ SEM. ($n = 20$). Asterisks indicated significant difference from control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ^a $P < 0.05$ indicated Jujul + NA versus Jujul. ^b $P < 0.05$ indicated Prazosin + NA versus Prazosin. ^c $P < 0.05$ indicated Prazosin + Jujul versus Jujul (ANOVA followed by Dunnett's test).

freely, but showed the decrease in sleep latency and the increase in the pentobarbital-induced sleeping time, similar to the results of diazepam (Figure 1B).

As we known, the multifaceted interaction of NA-, DA-, 5-HT-, ACh-, and histaminergic systems are required for the generation and maintenance of waking. According to diverse single-unit recording studies, the firing rates of these nuclei have been

described as high during waking, whereas their activity diminish during SWS and become completely silent across REM sleep [5]. Moreover, during the resting period, the activity of brain areas, such as neurons in the ventrolateral preoptic nucleus which contain the inhibitory neurotransmitter GABA, is enhanced, then innervate the ascending arousal system, including the locus coeruleus, dorsal raphe nucleus, ventral periaqueductal gray, tuberomammillary nucleus, and parabrachial nucleus, in turn, inhibit the activity of these waking systems [6].

Therefore, to explore further the possible mechanism of hypnosis, we examined the effects of histamine, NA, DA, 5-HT, ACh, and d-bicuculline on the sedative activity of Jujul. Mice were treated with Jujul (40 mg/kg, i.p.), the vehicle (10 mL/kg, i.p.), histamine (3.5 mg/kg, i.p.), NA (0.2 mg/kg, s.c.), DA (2.5 mg/kg, i.p.), 5-HT (1 mg/kg, i.p.), ACh (5 mg/kg, s.c.), or d-bicuculline (5 mg/kg, i.p.), respectively, 15 min or 25 min before pentobarbital sodium was injected. In combination, mice were treated with Jujul or the vehicle 10 min after histamine, NA, DA, 5-HT, ACh, or d-bicuculline was administered. Fifteen min later, pentobarbital sodium was injected. As describe previously, T₁, T₂, and T₃ were observed, respectively. Interestingly, DA, 5-HT, ACh, histamine, or NA could not influence the pentobarbital sodium-induced sleep time in T₂ when use alone, in spite that they enhanced sleep latency (T₁). However, in combination with Jujul (40 mg/kg), only NA could effectively weaken the sedative activity of Jujul in T₂. Although d-bicuculline shortened T₂ and increased T₁ when use alone, it could not antagonize the hypnosis of Jujul in T₂ (Table 1). Those data indicated that the hypnosis of Jujul was not related to histamine receptors, dopaminergic receptors, serotonergic receptors, acetylcholine receptors or GABA_A receptors, but might involve in the adrenoceptors.

It is well known that norepinephrine is one of the main neurotransmitters involved in arousal. The locus coeruleus neurons fire in a wake-dependent manner; they are highly active during wake, slow-firing during nonREM sleep, and almost completely quiescent during REM sleep [7]. In addition, the $\alpha 1$ brainstem noradrenergic nuclei also provide input to regions of the hypothalamus which are known to be involved in sleep regulation [8]. Several lines of evidence have shown that the suppression of LC activity leads to sedation and drives forebrain EEG recordings into sleep-like patterns [9]. To demonstrate deeply the possible mechanism, 15 min or 25 min before pentobarbital sodium was injected, mice were treated with Jujul (20 mg/kg, i.p.), the vehicle (10 mL/kg, i.p.), or prazosin (2 mg/kg, i.p.), respectively. In combination, mice were treated with Jujul 10 min after prazosin, or NA (0.2 mg/kg, s.c.) was administered intraperitoneally. Fifteen min later, pentobarbital sodium was injected. T₁, T₂, and T₃ were observed. In addition, mice treated with prazosin (2 mg/kg, i.p.) were also administered intraperitoneally with NA in advance, and T₁, T₂, T₃ were observed. The data showed that when Jujul (20 mg/kg) was coadministered with prazosin (2 mg/kg), both of them could show the synergic effect on T₁ and T₂ in the pentobarbital sodium-induced sleep time test. However, NA could antagonize effectively the sedative activity of Jujul or prazosin (Figure 1C). It was confirmed further that Jujul might block NA binding to $\alpha 1$ -adrenoceptors on the neuron membrane, in turn, weaken the fire rate of these neurons, leading to the decrease in latency of sleep and the enhancing of sleeping time.

Table 1 Effects of Jujubasaponins co-administered with different drugs on pentobarbital sodium-induced sleep time

Drug	Dosage	T ₁ (min)	T ₂ (min)	T ₃ (min)
Vehicle	10 mL/kg, i.p.	4.56 ± 0.30	37.2 ± 3.46	13.8 ± 3.29
Jujul	40 mg/kg, i.p.	3.45 ± 0.40*	61.0 ± 4.34***	17.4 ± 2.65
Histamine	3.5 mg/kg, i.p.	6.21 ± 0.64*	31.4 ± 4.00	11.4 ± 1.78
5-HT	1 mg/kg, i.p.	5.58 ± 0.35*	30.4 ± 3.20	14.1 ± 2.91
ACh	5 mg/kg, s.c.	6.37 ± 0.60**	28.0 ± 3.81	12.0 ± 1.59
d-bicuculline	5 mg/kg, i.p.	7.58 ± 0.49***	24.7 ± 2.49**	17.5 ± 1.86
D A	2.5 mg/kg, i.p.	5.79 ± 0.44*	32.5 ± 3.72	16.9 ± 1.54
N A	0.2 mg/kg, s.c.	5.84 ± 0.49*	30.5 ± 3.09	16.2 ± 2.77
Jujul + Histamine	40 mg/kg + 3.5 mg/kg	6.24 ± 0.72 ^a	55.3 ± 4.48**	10.1 ± 2.52
Jujul + 5-HT	40 mg/kg + 1 mg/kg	6.15 ± 0.56 ^a	56.0 ± 5.48**	20.4 ± 3.01
Jujul + ACh	40 mg/kg + 5 mg/kg	5.93 ± 0.56 ^a	54.7 ± 6.51*	13.2 ± 2.69
Jujul + d-bicuculline	40 mg/kg + 5 mg/kg	6.27 ± 0.49 ^{a,b}	50.4 ± 3.37*	12.4 ± 3.18
Jujul + D A	40 mg/kg + 2.5 mg/kg	5.94 ± 0.54 ^a	52.6 ± 4.62*	10.8 ± 3.26
Jujul + N A	40 mg/kg + 0.2 mg/kg	5.86 ± 0.48 ^a	40.4 ± 2.60 ^b	18.2 ± 4.18

Mice were treated with Jujul (40 mg/kg, i.p.), the vehicle (10 mL/kg, i.p.), histamine (3.5 mg/kg, i.p.), NA (0.2 mg/kg, s.c.), DA (2.5 mg/kg, i.p.), 5-HT (1 mg/kg, i.p.), ACh (5 mg/kg, s.c.) or d-bicuculline (5 mg/kg, i.p.), respectively, 15 min or 25 min before pentobarbital sodium was injected. In combination, mice were treated with Jujul or the vehicle 10 min after histamine, NA, DA, 5-HT, ACh or d-bicuculline was administered. Fifteen min later, pentobarbital sodium was injected. T₁: sleep latency, T₂: sleeping time, T₃: movement convalescence time. Each group represents the mean ± SEM. (n = 20). Asterisks indicated significant difference from control, *P < 0.05, **P < 0.01, ***P < 0.001. a, b, indicated significant between jujul and jujul + tool drugs, ^aP < 0.01, ^bP < 0.001 (ANOVA followed by Dunnett's test).

In conclusion, the study demonstrated the sedative activity of Jujul in the animal model of pentobarbital-induced sleep time and further suggested that the possible mechanism might be involved in $\alpha 1$ -adrenoceptors.

Conflict of Interest

The authors declare no conflict of interest.

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