Tranquilizer-like Effects of Sanjoinine A: Possible GABA/Benzodiazepine Receptors Complex Involvement

Yuan Ma[1], Jae Soon Eun[2], Ki-Wan Oh[3]

[1] *Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju. 361-763,*
[2] *College of Pharmacy, Woosuk University, Samrye, 565-701,*
[3] *College of Pharmacy, Chungbuk National University, Cheongju 361-763 Korea*

Zizyphi Spinosi Semen (ZSS) has been widely used for the treatment of anxiety and insomnia in Korea and China. This experiment was performed to know whether sanjoinine A, one of major alkaloid compounds of ZSS has anxiolytic and hypnotic effects through the GABAergic systems. Our results showed that administration of sanjoinine A increased open arm entries and spent time in open arm in the elevated plus-maze and increased head dips in hole board test. Different from traditional anxiolytic, diazepam, sanjoinine A itself did not decrease locomotor activity and strength level in mice. Furthermore, Sanjoinine A (0.5-2.0 mg/kg) prolonged sleeping time and reduced sleeping latency induced by pentobarbital in a dose-dependent manner similar to muscimol, a $GABA_A$ receptor agonist. Sanjoinine A (0.25-1.0 mg/kg) also increased sleeping rate and sleeping time in the combined administration at the sub-hypnotic dose of pentobarbital and showed synergic effects with muscimol in potentiating sleeping onset and enhancing sleeping time induced by pentobarbital. However, sanjoinine A itself did not induce sleeping at the higher dose. In addition, both of sanjoinine A and pentobarbital increased chloride influx in primary cultured cerebellar granule cells. Sanjoinine A decreased the $GABA_A$ receptor α-subunit expression and increased γ-subunit expression, and had no effects on abundance of β-subunit in primary cultured cerebellar granule cells, showing different expression of subunits from pentobarbital. In conclusion, sanjoinine A shows anxiolytic-like effects and augments pentobarbital-induced sleeping behaviors through the modification of GABAergic systems. [This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (The Regional Research Universities Program/Center for Healthcare Technology Development)].

Key words: Zizyphi Spinosi Semen (ZSS); Sanjoinine A; anxiolytic; sleep; pentobarbital; $GABA_A$ receptors

Tranquilizer-Like Effects of Sanjoinine A

- Modulation on GABAergic System -

Ma Yuan, Han Huishan, Oh Ki-Wan

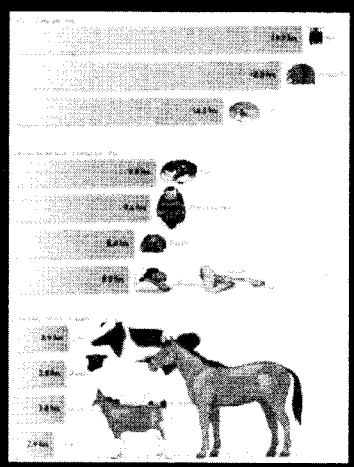
College of Pharmacy, Chungbuk National University, Cheongju 361-763 Korea

Sleep: Basic skill of many species

Sleep: Repair and Restoration

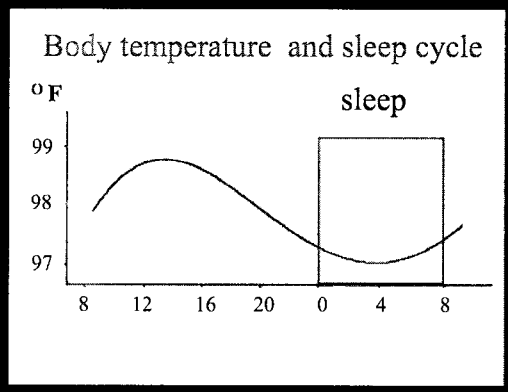
- We evolved to sleep so that we would conserve energy when we were least efficient

-Species will sleep different amounts depending on how much they must look for food and watch for predators



Characteristics of sleep

- Circadian rhythm
- Normal sleep patterns (cycles)
- Non REM
- REM

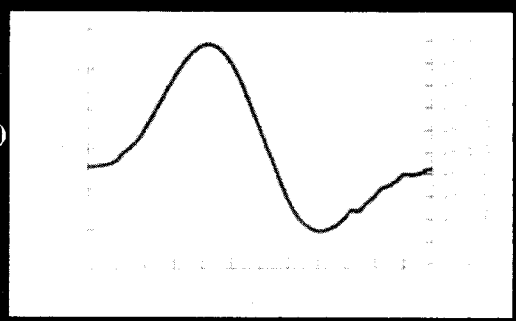


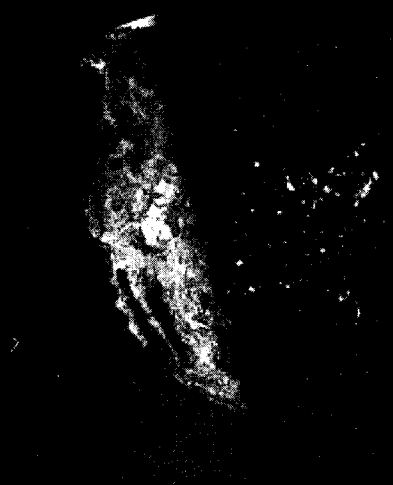
Circadian rhythms

Endogenous circadian rhythms

- **rhythms that last about a day**
- **humans' last around 24.2 h**

Examples:
- **activity (sleeping and waking)**
- **temperature**
- **eating and drinking**
- **secretion of hormones**





Difficulties with sleep are commonly found among 20-38% of the population in various countries

Bixler EO *et al. Am J Psychiatry* 1979; 136: 1257- 1262.

Klink M, Qun SF. *Chest* 1987; 91: 540–546.

Mellinger GD, Balter MB, Uhlenhuth EH. *Arch Gen Psychiatry* 1985; 42: 225 -232.

Ohayon M. *Sleep* 1996; 19: S7–S15.

Status in Americans

- 62% say the experience sleep problems a week or more;
- 40% report sleepy enough during the day to interfer with activities;
- 62% drive while drowsy;
- 27% have fallen asleep while driving;
- 60% children feel parients are tired during the day;
- 15% children admit falling asleep at school.

Status in men and women

Man

- On week days one third of men get less than 6 hours sleep
- 47% truck drivers report falling asleep at the wheel
- 25% of truck drivers fall asleep yearly

Women

- 79% report sleep disturbance during pregnancy
- 36% women peri- menopausal women report disturbed sleep
- 25% suffer from significant daytime sleepiness
- 30% disturbed sleep interferes with daily activity, 27% job performance is impaired, 24% say sleep gets in the way of caring for family

Insomnia

- Difficulty initiating sleep
- Frequent awakenings (including early morning awakening)
- Subjective feeling of insufficient sleep
- Preoccupation with sleep complants

Types of insomnia

- Short-term
  - Last from a few nights to a few weeks;
  - Caused by worry over a stressful situation .
- Long-term (chronic)
  - Last months or even years;
  - Caused by general anxiety, medications, chronic pain, etc...

Causes of insomnia

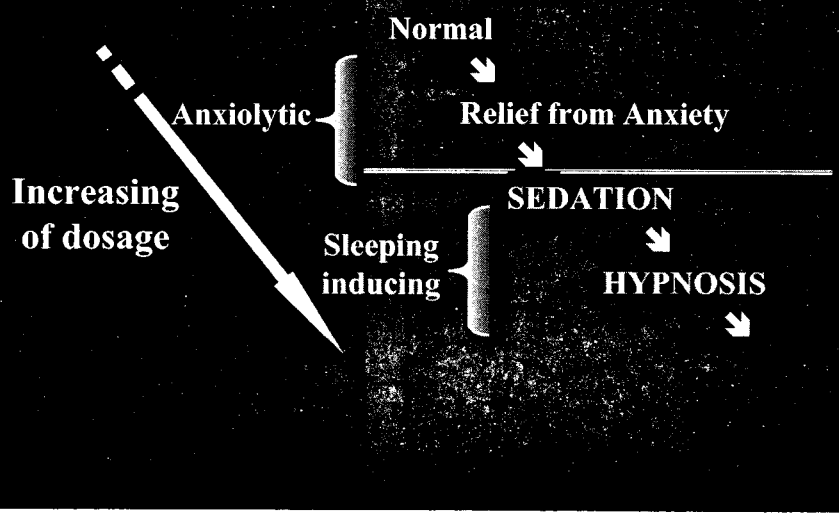
- Stress
- Fear
- Anger
- Depression
- Behavioral factors
- Life-style
- Personality
- Medicine

} Stressful and anxiety related events

Treatment of insomnia

- Nonpharmacologic treatment
  - Hygiene
  - Relaxation
  - Stimulus control
  - Sleep restriction
- Pharmacologic therapy
  - Benzodiazepines/related hypnotics
  - Antidepressants
  - Antihistamines
  - Melatonin

Tranquilizers



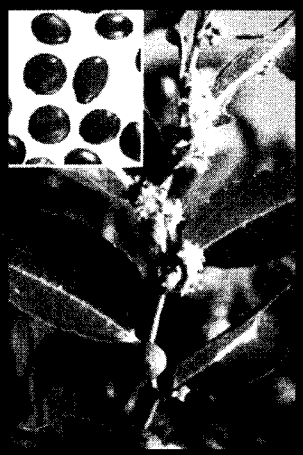
Side effects of traditional tranquilizers

- Architecture of sleep disruption;
- CNS depression, include: drowsiness, excess sedation, impaired coordination, nausea, vomiting, confusion and memory loss;
- Tolerance develops;
- Dependence may develop;
- Serious withdrawal syndrome characterized by increase anxiety, insomnia, CNS excitability and convulsions. can include convulsions and death;
- Drug abuse;
- No medication against overdose effects.

Herbal remedies for insomnia

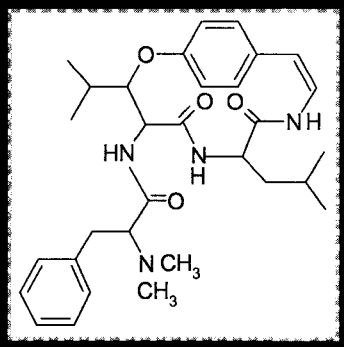
- **Chamomile (*Anthemis nobilis*):** Commonly used in Europe, South America, and Mexico for insomnia and restlessness combined with irritability, particularly in children.
- **Hops (*Humulus lupulus*):** A major flavoring component of beer, has a long history of use for sleeplessness, nervousness, and restlessness. Hops pillows are sometimes used for mild insomnia.
- **Lavender (*Lavandula officinalis*):** Recommended for persons with sleep disorders, may be used as a compress or massage oil or simply inhaled to alleviate insomnia.
- **Passion flower (*Passiflora incarnata*):** An important herb for insomnia caused by mental worry, overwork, or nervous exhaustion. It is an excellent sedative with no side effects even when used in large doses (Spaick, 1978).
- **Valerian (*Valeriana officinalis*):** It shortens sleep latency and reduces nighttime waking. It works well in combination with other sedative herbs, such as California poppy, skullcap, hops, and passion flower.
- **Wild lettuce (*Lactuca virosa*):** A mild sedative and nervine used for restlessness and insomnia. It may be found in a variety of formulas for the treatment of acute and chronic insomnia.
- **California poppy (*Eschscholzia californica*):** Has mild sedative and analgesic properties, can be given safely to children, has been shown to improve both sleep latency and quality (Bruneton, 1995).
- **Kava-kava (*Piper methysticum*):** This sedative herb is often used for sleeplessness and fatigue.
- **St. John's wort (*Hypericum perforatum*):** Is quickly becoming an important part of modern herbal therapeutics, it can help relieve chronic insomnia and mild depression when related to certain brain chemistry imbalances.
- **Herbal formulas for insomnia**

Zizyphi Spinosi Semen



- Zizyphi Spinosi Semen (ZSS), the dried seed of *Zizyphus jujuba Mill var. spinosa* (Rhamnaceae);
- Has been used as a tranquilizer, an analgesic and an anticonvulsant in oriental countries such as Korea and China for centuries;
- Has been prescribed for the treatment of insomnia and anxiety in Asia.

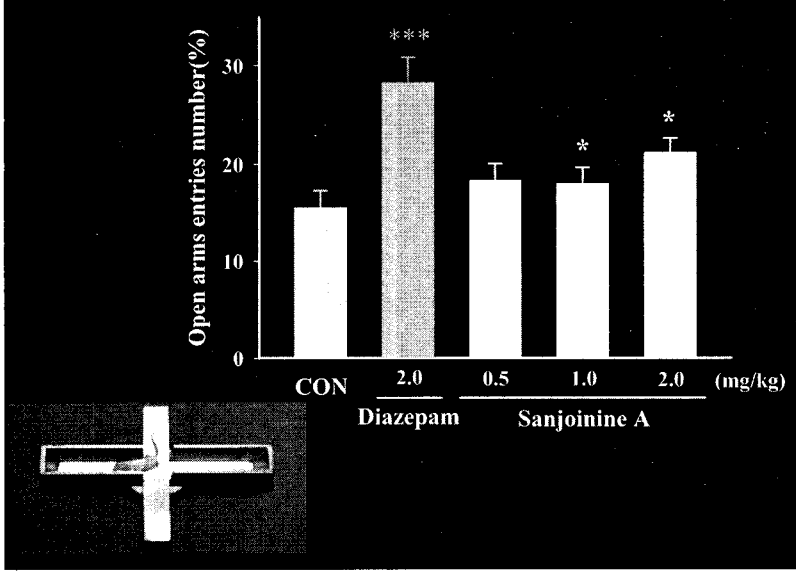
Structure of sanjoinine A

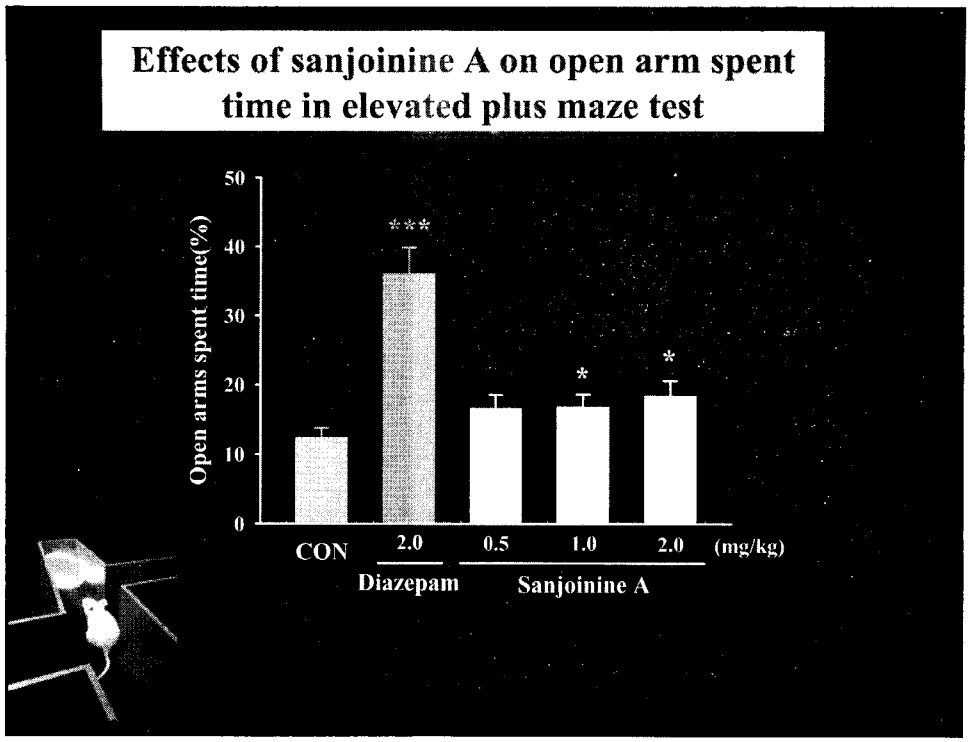


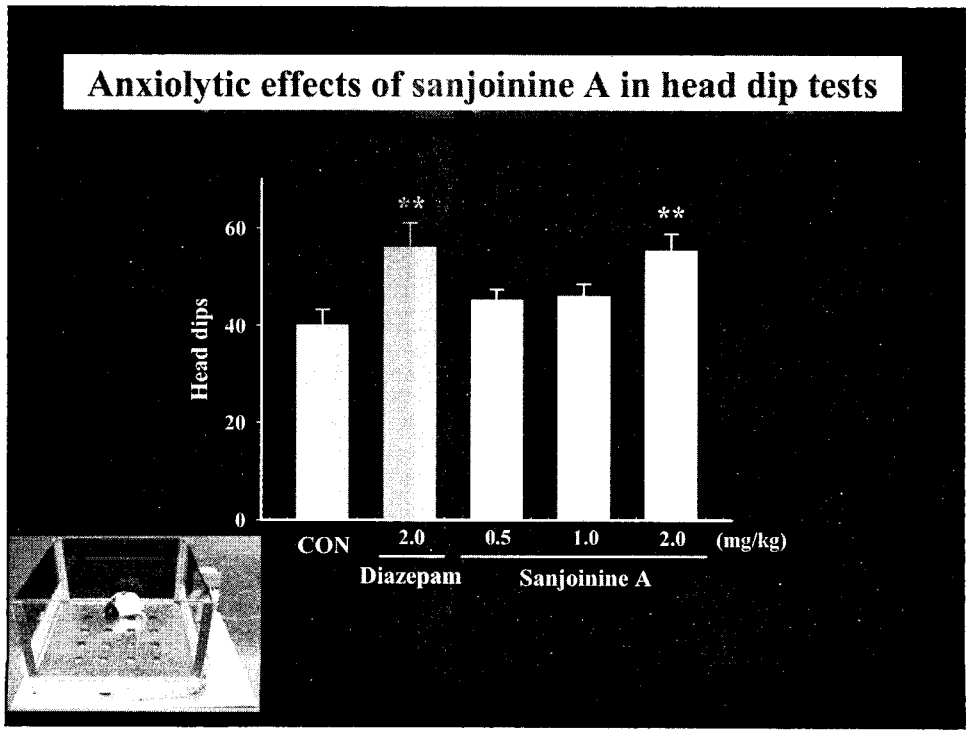
- Sanjoinine A, one of the cyclopeptides from ZSS;
- Demonstrated to be the major hypnotic component of ZSS.

Anxiolytic effects of sanjoinine A

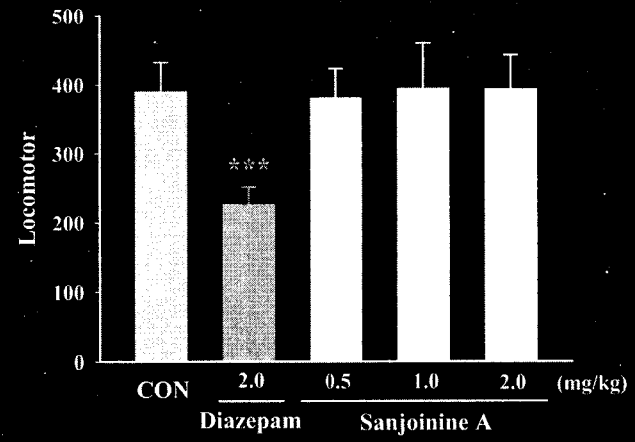
Effects of sanjoinine A on open arm entries in elevated plus maze test



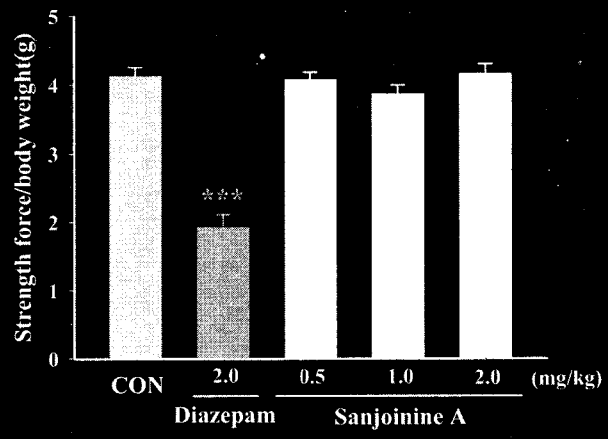

Effects of sanjoinine A on open arm spent time in elevated plus maze test
Open arms spent time(%)
50
40
30
20
10
0
***
*
*
CON
2.0
0.5
1.0
2.0
(mg/kg)
Diazepam
Sanjoinine A


Anxiolytic effects of sanjoinine A in head dip tests
Head dips
60
40
20
0
**
**
CON
2.0
0.5
1.0
2.0
(mg/kg)
Diazepam
Sanjoinine A

Effects of sanjoinine A on locomotor activity in mice

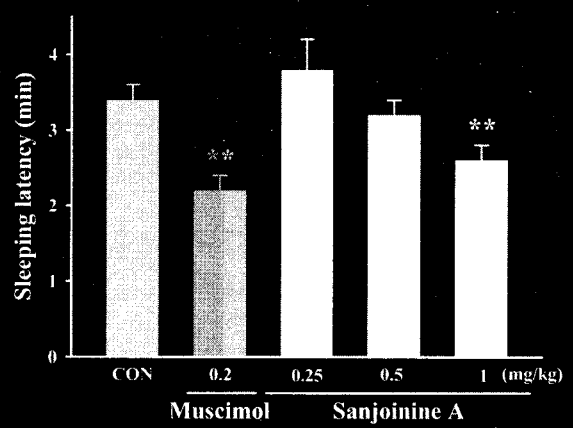


Effects of sanjoinine A on strength force in mice

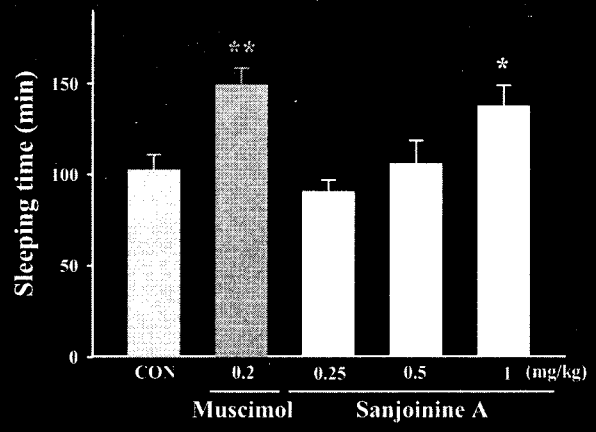


Sedative/hypnotic effects of sanjoinine A

Effects of sanjoinine A on sleep latency of pentobarbital-induced sleeping behaviors



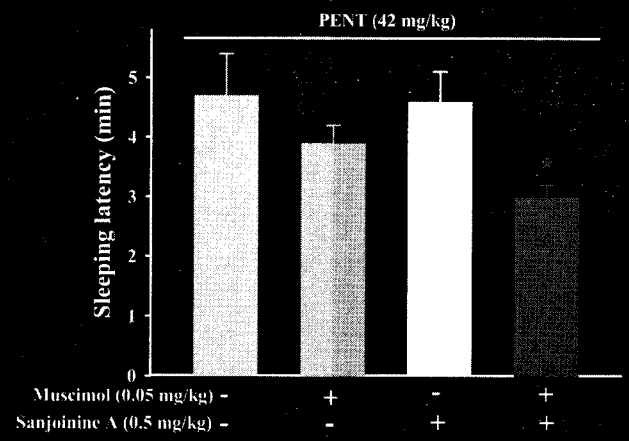
Effects of sanjoinine A on sleep time of pentobarbital-induced sleeping behaviors



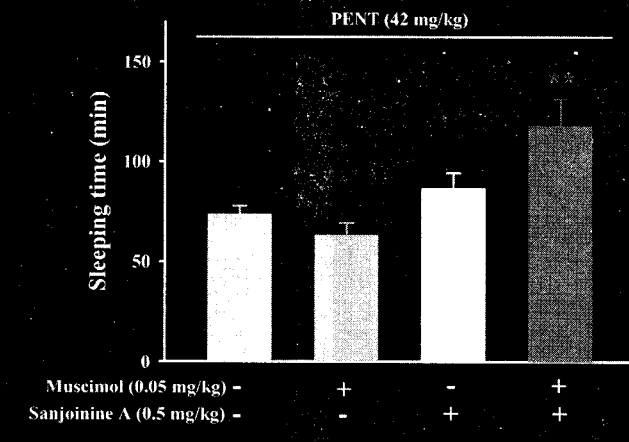
Effects of sanjoinine A on sleeping onset of mice treated by sub-hypnotic dose of pentobarbital (28 mg/kg, i.p.)

Group	Dose (mg/kg)	No. falling asleep/total	Sleeping time (min)
Control	-	5/15	13.8±5.4
Muscimol	0.2	12/15 *	55.4±11.4 **
Sanjoinine A	0.25	6/15	13.9±4.9
	0.50	8/15	18.6±5.2
	1.00	13/15 **	65.3±10.7 **

Synergic effects of sanjoinine A with muscimol on pentobarbital-induced sleeping behaviors



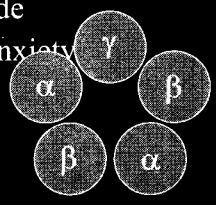
Synergic effects of sanjoinine A with muscimol on pentobarbital-induced sleeping behaviors



Possible mechanisms of sanjoinine A

The GABA inhibitory system

- GABA-mediated inhibition is found in all brain regions
- $GABA_A$ receptor subtype, a member of a superfamily of ligand-gated ion channels.
  - 5 separate protein subunits
  - at least 16 genes for types of subunits
  - passage of negatively charged iron: chloride
  - reduce the cell's excitability =>decrease anxiety

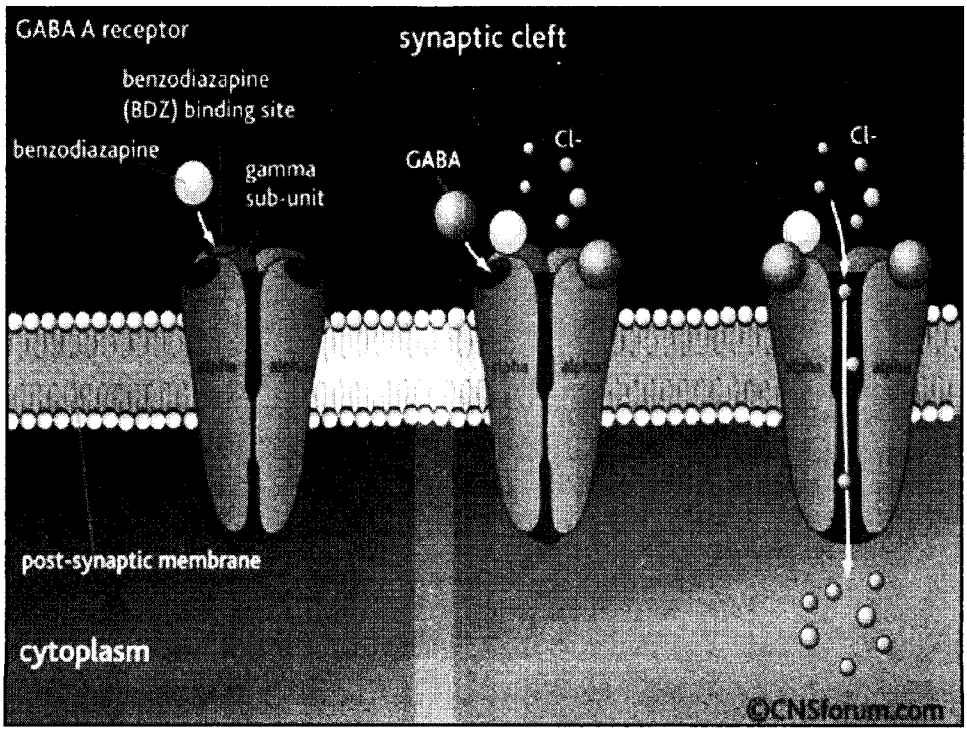


Transquilizer (Anxiolytic/Sedative/Hypnotic)

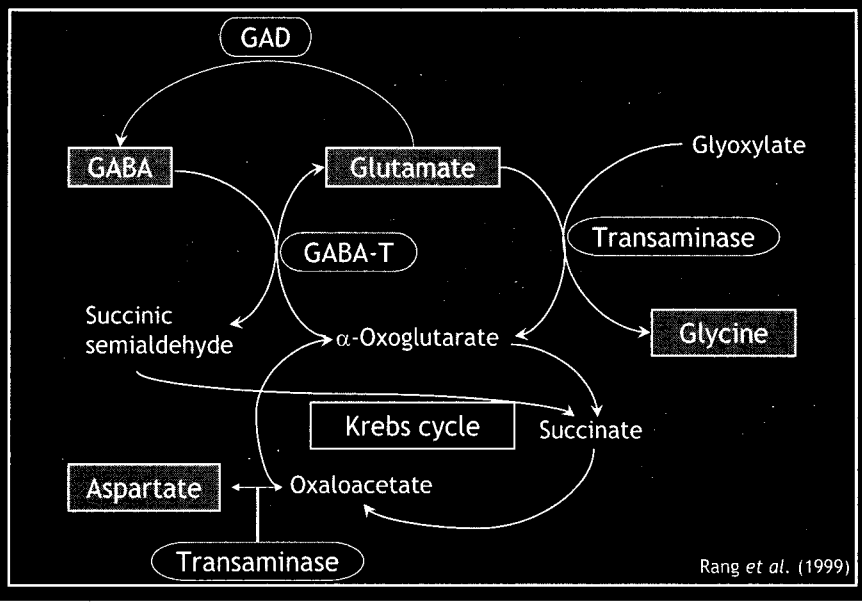
BENZODIAZEPINES

BARBITURATES

GABAergic system



Transmitter amino acids in brain

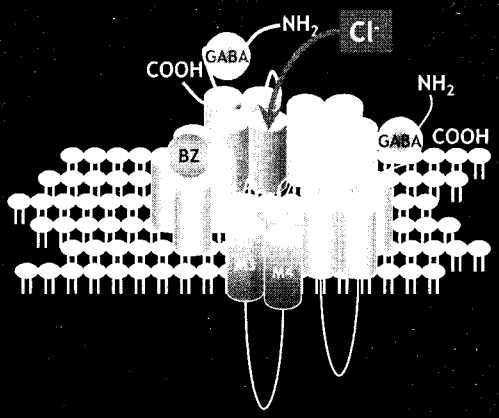


$GABA_A$ subunits

- $GABA_A$ receptor is made up of subunits (α,β,γ,δ) that occur in different combinations in various parts of the brain —change with location.
- $GABA_A$ receptor and the subunits differ at specific developmental stages —change over time.
- These differences allow binding for different drugs and determine subsequent pharmacological activity.
- As a result of the above, AEDs may have different effects in the neonatal brain as compared to the adult brain

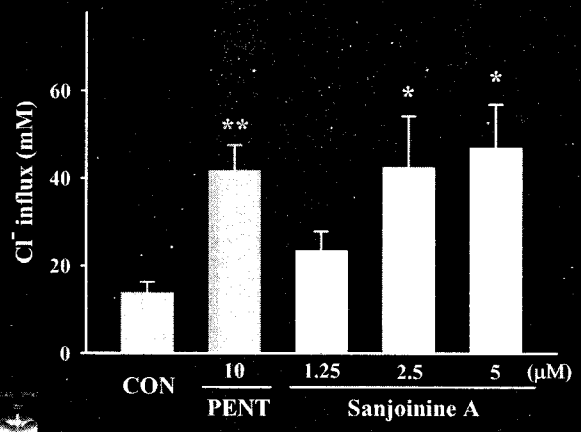
Ann Pharmacother 2002;36(7-8):1150-5

$GABA_A$ receptors



Physiol. Rev. (2002) 82, 503-568.
Prog. Neurobiol. (2002). 67, 113-159.
J. Psychiatry Neurosci. (2003). 28, 263-274.

Effects of sanjoinine A on chloride influx in primary cultured cerebellar neurons



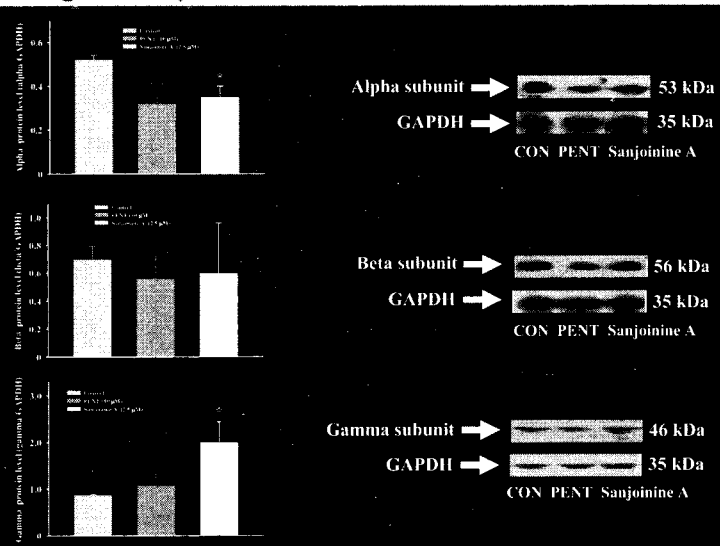
$GABA_A$ receptors

Structural subtypes

1. $GABA_A$ Receptors
   a. 5 Subunits;
   b. 13 Subunits subcategories
   c. Over 74000 possible combinations
   d. Not all subcategories known
2. GABAR subunit expression differs at a regional and cellular level, where it determines the functional and pharmacological properties of the receptor.

Rudolph U, Crestani F, Benke D, Brunig I, Benson J, Fritschy J, Martin J, Bluethmann H, Mohler H (1999) Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 401:796–800.

Effects of sanjoinine A on $GABA_A$ receptor in primary cultured cerebellar neurons



Glutamic acid decarboxylase (GAD)

Alpha-Ketoglutarate

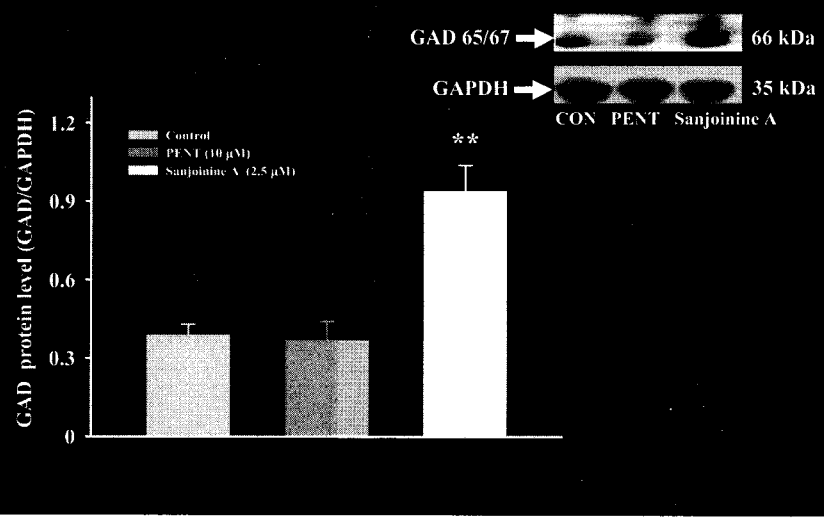
↓ GABA-oxoglutarate transaminase (GABA-T)

Glutamate (Excitatory)

↓ **GAD**

GABA (Inhibitory)

Effects of sanjoinine A on GAD 65/67 in primary cultured cerebellar neurons



Results (1)

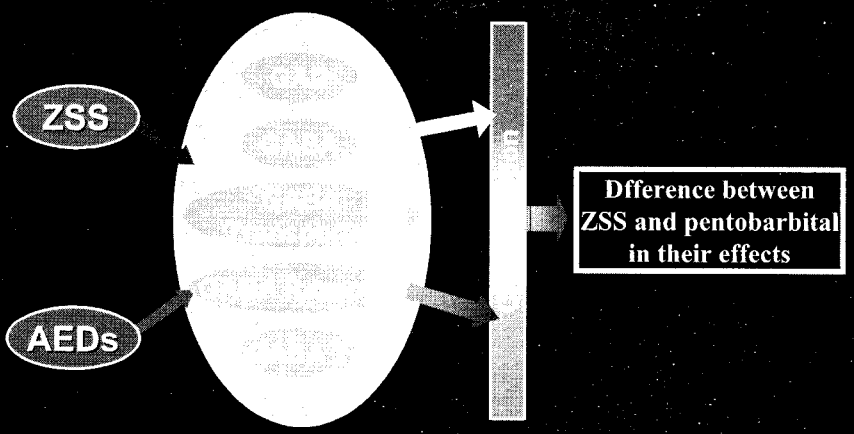
- Sanjoinine A has anxiolytic effects.
- Sanjoinine A prolonged sleeping time and reduced the sleeping latency induced by pentobarbital.

Results (2)

- Sanjoinine A shows synergistic effects with muscimol in potentiating pentobarbital induced sleeping behavior;
- Sanjoinine A increases Cl- influx;
- Sanjoinine A changes the $GABA_A$ receptor subunit expression, showing different expression of subunits from pentobarbital;
- Sanjoinine A increases GAD expression.

Conclusions

- Sanjoinine A shows anxiolytic effects without changing locomotor activity and strength levels;
- Sanjoinine A augments pentobarbital-induced sleeping behaviors through the modification of GABAergic systems.
- GABAergic systems may be one of targets by which sanjoinine A exert its tranquilizer-like effects.



Differences in $GABA_A$ receptor subunits expression, together with changes in GAD may elicit distinct responses to traditional hypnotics, such as pentobarbital.

Thanks for your attention!