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Selective Depleting Effect of Syrosingopine on Brain Catecholamine Levels with Relation to Morphine Analgesia in the Rat

T. FURUKAWA[1], T. SANO, Y. KOHNO, M. KOGA AND N. NAGASAKI

Department of Pharmacology, Kurume University School of Medicine, Kurume, Japan

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FURUKAWA, T., T. SANO, Y. KOHNO, M. KOGA AND N. NAGASAKI. *Selective depleting effect of syrosingopine on brain catecholamine levels with relation to morphine analgesia in the rat.* PHARMAC. BIOCHEM. BEHAV. 4(4) 419–425, 1976. – Reserpine was the most potent, rescinnamine the next and syrosingopine the weakest in the depleting effects on brain amines of rauwolfia alkaloids. After syrosingopine, brain dopamine (DA) was decreased to a smaller degree and with a shorter duration as compared with norepinephrine (NE) and serotonin (5-HT), whereas reserpine elicited a marked and long lasting reduction in these amines. Accordingly, syrosingopine induced a depletion of brain NE and 5-HT without alteration in brain DA content 2–4 days after administration. Repeated administrations of syrosingopine, 2 mg/kg daily for 2 or 4 days, resulted in similar alterations in brain amine levels. This selective depleting effect of syrosingopine on brain amines was potentiated by combined treatment with disulfiram or fusaric acid, a dopamine β-hydroxylase inhibitor. Under the condition of selective depletion of brain amines induced by repeated administrations of syrosingopine, 2 mg/kg daily for 2 days, the analgesic action of morphine was not affected, whereas reserpine and tetrabenazine antagonized morphine analgesia, concomitant with inducing a depletion of all brain amines. The results suggest that brain DA may be more important than brain NE or 5-HT with regard to the mechanisms by which morpine produces analgesia.

Syrosingopine Brain amine Selective depletion Dopamine Morphine analgesia

SYROSINGOPINE is a synthetic analogue of reserpine and has been proposed to be considerably less potent centrally than is reserpine though it has approximately the same degree of activity at peripheral sites. It is reported that depressor and negative chronotropic effects of syrosingopine are similar to those of reserpine while central effect, sedation, is 1/20–1/40 that of reserpine [10,28]. Syrosingopine exhibits peripheral action in doses that does not induce the central actions [26]. Meanwhile, reserpine and related alkaloids are known to deplete stores of endogenous amines in various tissues [14].

The present study was undertaken to investigate the effect of syrosingopine in depleting endogenous amines and to study the relationships between syrosingopine-induced selective depletion of brain amines and morphine analgesia.

METHOD

Animals

Animals in the experiment were healthy Wistar male rats (180–200 g) obtained from Kuroda Animal Laboratory (Kumamoto, Japan). They were housed in groups of 5 for a week before as well as throughout the experiment and were always placed with their cagemates after injection or between test trial. The food consisted of CE-2, CLEA, Japan, and the animals were permitted food and water ad lib. All trials and breedings were carried out at an environmental temperature 24 ± 1°C and moisture 50 ± 10%.

Procedure

Assaying the endogenous amine. Rats were sacrificed by decapitation, and the brain, heart and adrenals were rapidly removed. These tissues were stored frozen at −20°C, and, then, monoamine contents in the tissues were determined within 4 days. The brain was divided into 2 cerebral hemispheres after removal of cerebellum. One hemisphere was taken for 5-HT analysis and the other for catecholamines analysis. Both adrenal glands were taken for catecholamine determinations after removing their capsules. Catecholamines and 5-HT were determined by the methods described by Anton, *et al.* [1,2] and Bogdanski, *et al.* [4] respectively, with minor modifications. The fluorescence intensity was measured with a recording spectrofluorophotometer (Simadzu RF 501).

Assessing the analgesic effect of morphine. The antinociceptive activity of morphine was determined using the vocalization test according to the method described by Carroll and Lim [5], whereby vocalization was elicited by

[1] Present address: Department of Pharmacology, School of Medicine, Fukuoka University, Fukuoka 814, Japan.

electrical stimulation of the tail. In this experiment, the mean threshold which caused vocalization during stimulation was 0.66 ± 0.14 volt in normal rats, and, after morphine, the voltage of electrical stimulation was increased to obtain the threshold as follows; control voltage(V) × (1 + 0.1), V × $(1 + 0.1)^2$ ..... V × $(1 + 0.1)^n$.

Drugs

The drugs used in this study were prepared as follows before each administration to rats; reserpine, rescinnamine and syrosingopine (Inverni and Della) were dissolved in a 20% ascorbic acid solution. Disulfiram (Nokkubin, Tōkyo Tanabe) was suspended in saline. Fusaric acid (Banyū Pharmaceutical) was dissolved in 0.01N HCl and adjusted pH to 6.0 with 0.4N NaOH. Tetrabenazine hydrochloride (F. Hoffman-La Roche) was dissolved in distilled water. These drugs were injected intraperitoneally to experimental animals.

Statistical Analysis

Tissue amine concentrations were expressed as mean values ± standard error of the mean (μg/g tissue) and statistical analysis was calculated using the Student's *t*-test.

RESULTS

Dose-response Studies on Effects of Three Rauwolfia Alkaloids on Amine Contents

Monoamine contents were determined 24 hr after a single administration of various doses of syrosingopine, rescinnamine and reserpine. Control values of brain DA, NE and 5-HT levels obtained from untreated rats were 1.017 ± 0.020 μg/g (n = 38), 0.424 ± 0.014 μg/g (n = 36) and 0.546 ± 0.012 μg/g (n = 33) respectively. When the animals received the same volumes of vehicle used for the alkaloids, monoamine contents were not affected. As shown in Fig. 1, in the depleting effect on brain amines, syrosingopine was the weakest, monoamine contents being reduced significantly by only a large dose of 10 mg/kg. Rescinnamine was the next. Reserpine was the most potent and produced marked dose-dependent reductions of all monoamines even in relatively small doses of 1 and 2 mg/kg.

The level of heart NE was 0.883 ± 0.042 μg/g (n = 29) in the untreated rats and was lowered markedly by each alkaloid at a dose of 1 mg/kg.

Adrenal DA, NE and epinephrine contents in the untreated rats were 4.90 ± 0.29 μg/g (n = 22), 221.13 ± 17.71 μg/g (n = 22) and 535.06 ± 29.52 μg/g (n = 22) respectively. The adrenal DA was resistant to the depleting action of any of the 3 rauwolfia alkaloids and was increased slightly instead after syrosingopine or rescinnamine, 1 or 2 mg/kg. NE and epinephrine contents tended to be reduced by syrosingopine and rescinnamine, 1 or 2 mg/kg, though the differences from the untreated animals were not statistically significant. Reserpine, 1 or 2 mg/kg, decreased significantly both NE and epinephrine levels.

Time Course of Changes in Brain Amines Levels after Syrosingopine or Reserpine

In the following items of the experimental series, control values of brain DA, NE and 5-HT in untreated animals were 1.082 ± 0.020 μg/g (n = 75), 0.396 ± 0.010 μg/g (n = 77) and 0.548 ± 0.009 μg/g (n = 75) respectively.

The brain monoamine levels were determined at various time intervals after a single administration of syrosingopine, 5 mg/kg, or reserpine, 1 mg/kg.

The result is shown in Fig. 2. Both alkaloids elicited general decreases of brain monoamine which were maximum 4–24 hr after the administration. However, although the decreases in brain NE and 5-HT levels observed after syrosingopine were similar to those seen after reserpine, the decrease in brain DA level after syrosingopine was weaker and recovery from the decrease was faster as compared with those seen after reserpine.

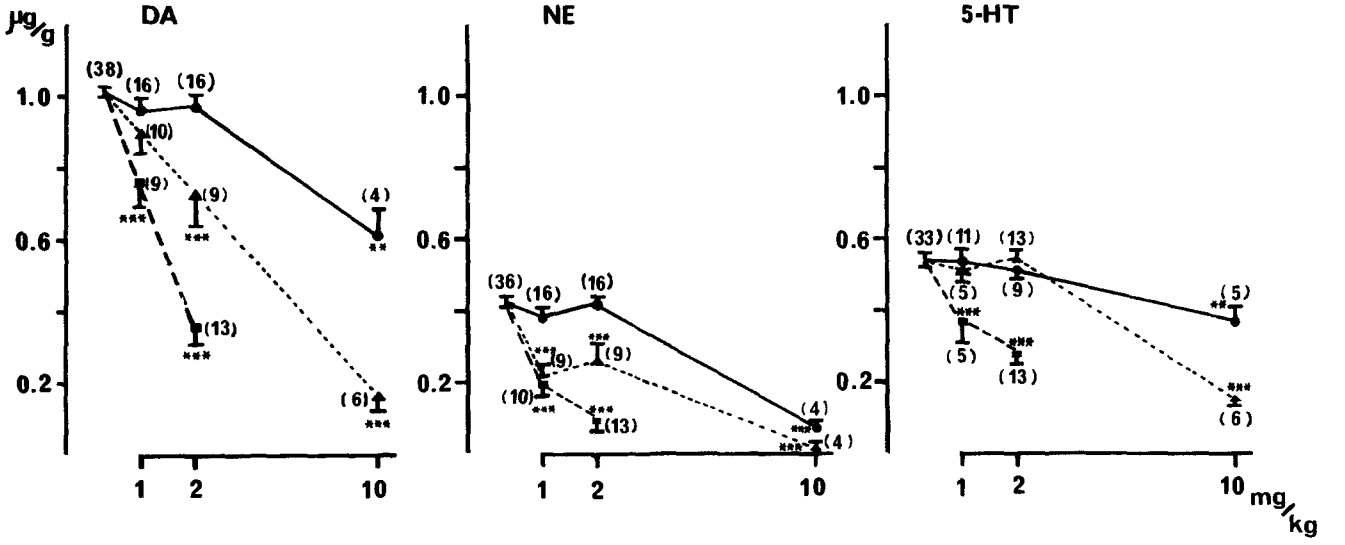

FIG. 1. Dose-responses of brain amine levels to rauwolfia alkaloids. Dopamine; DA, norepinephrine; NE, serotonin; 5-HT. ——●——; syrosyngopine, - - -▲- - -; rescinnamine, ——■——; reserpine. Number in parentheses; number of rats used. *; significant difference from the normal level (*; $p<0.05$, **; $p<0.01$, ***; $p<0.001$). Vertical bars; standard errors of the mean.

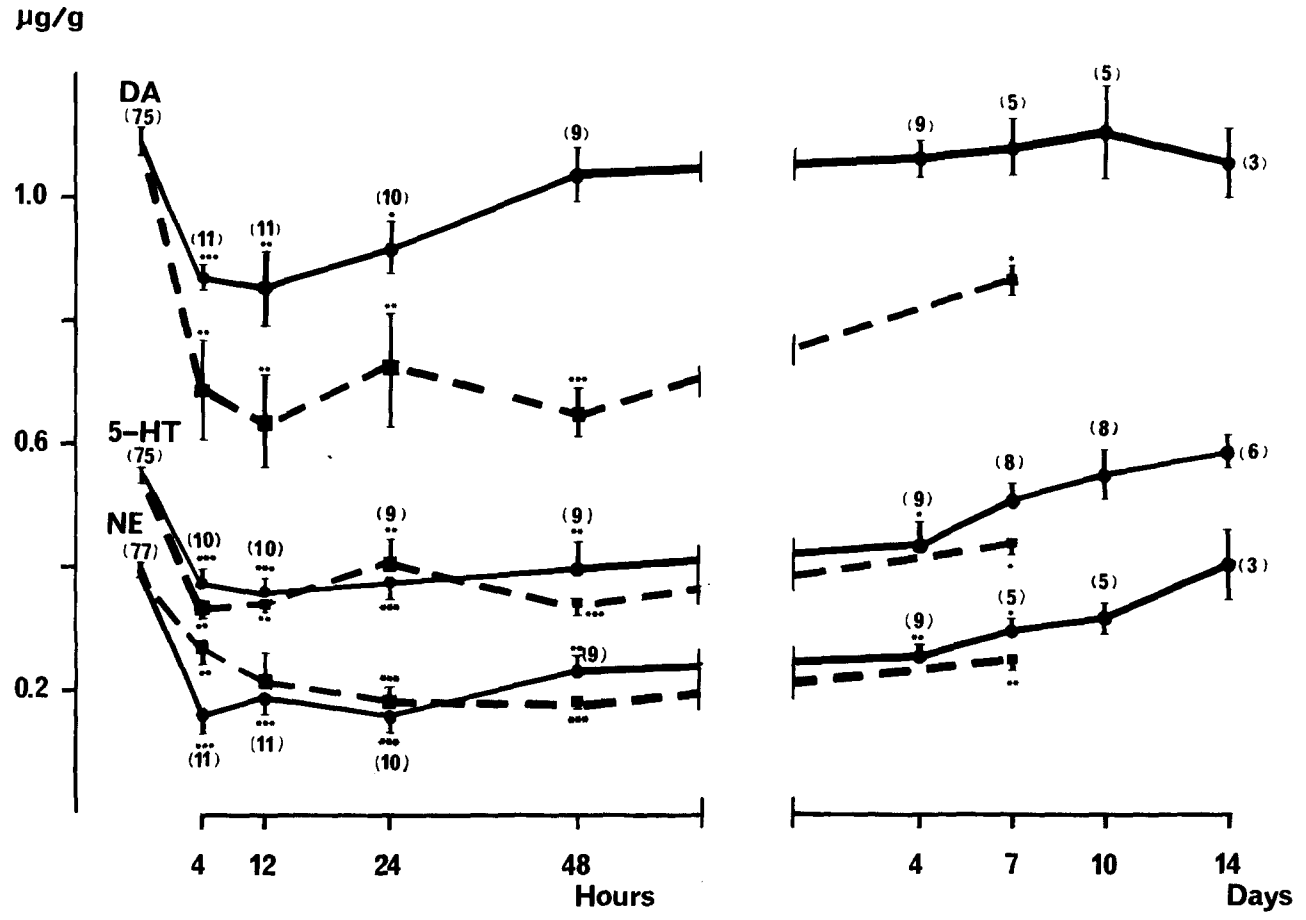

FIG. 2. Time courses of changes in brain amine levels after syrosingopine (5 mg/kg) or reserpine (1 mg/kg). Further explanations as in Fig. 1.

Therefore, 2–4 days after syrosingopine (5 mg/kg), brain NE and 5-HT were reduced whereas brain DA level was unchanged. All central amines were reduced at this time after reserpine (1 mg/kg).

Changes in Brain Monoamine Contents after Repeated Administrations of Syrosingopine

The effect of syrosingopine administered repeatedly on brain amines was investigated.

Rats were injected daily with different 5 doses (1, 2, 2.5, 3 or 4 mg/kg) of syrosingopine for 4 days and decapitated 24 hr after the final injection. As shown in Fig. 3, brain NE and 5-HT were reduced dose-dependently, while brain DA tended to be slightly increased with smaller doses (1 or 2 mg/kg) but decreased dose-dependently when larger daily doses (2.5, 3 or 4 mg/kg) were used. Thus the administrations of syrosingopine for 4 days resulted in a slight increase of brain DA content accompanied with decreases of brain NE and 5-HT contents at doses of 1 and 2 mg/kg but caused decreases of all brain amines at doses of 2.5, 3 and 4 mg/kg.

Syrosingopine or reserpine was administered daily for 2 days and the rats were decapitated 24 hr later. Tetrabenazine was injected once and the rats were decapitated 70 min later. As seen in Table 1, syrosingopine brought about significant decreases of NE and 5-HT contents without alterating DA content at a dose of 2 mg/kg whereas it induced decreases of all brain amines contents at a dose of 5 mg/kg. Reserpine (2 mg/kg for 2 days) and tetrabenazine (10 mg/kg) caused decreases of all 3 brain amines.

Influences of Disulfiram and Fusaric Acid on the Effect of Syrosingopine

Disulfiram (400 mg/kg) decreased brain NE in a time-dependent manner, 1, 2 and 6 hr after its administration but did not affect significantly either brain DA or 5-HT as demonstrated in Fig. 4. Syrosingopine (2 mg/kg) was administered daily for 2 days as described above and disulfiram (400 mg/kg) additionally 1 or 2 hr before decapitation. After the combined treatment with syrosingopine and disulfiram, a decrease in brain NE was potentiated.

After fusaric acid (75 mg/kg), brain DA tended to be increased, and brain NE was decreased significantly, whereas brain 5-HT was increased significantly 20 hr later. Syrosingopine (2 mg/kg) was injected similarly for 2 days and fusaric acid (75 mg/kg) additionally 20 hr before decapitation. Alterations in brain amine levels were generally similar to those seen by combined use of syrosingopine and disulfiram.

Influences of Rauwolfia Alkaloids on Morphine Analgesia

Influences of rauwolfia alkaloids on morphine analgesia were investigated under the conditions of the alkaloids-

TABLE 1

CHANGES IN BRAIN AMINE LEVELS BY SYROSINGOPINE, RESERPINE OR TETRABENAZINE

Drugs (mg/kg x Injection Times)	Brain Amines Contents (M ± SE, μ/g) Dopamine	Norepinephrine	Serotonin
Untreated	1.082±0.020 (n=75)	0.396±0.010 (n=77)	0.548±0.009 (n=75)
Syrosingopine (2 x 2)	1.112±0.043 (n=38)	0.234±0.010* (n=39)	0.460±0.023* (n=33)
Syrosingopine (5 x 2)	0.597±0.075* (n=5)	0.235±0.027* (n=5)	0.370±0.037* (n=5)
Reserpine (2 x 2)	0.114±0.020* (n=4)	0.021±0.003* (n=4)	0.183±0.021* (n=4)
Tetrabenazine (10 x 1)	0.128±0.023* (n=6)	0.082±0.023* (n=6)	0.357±0.049* (n=6)

Syrosingopine (2 or 5 mg/kg) and reserpine (2 mg/kg) were administered daily for 2 days and tetrabenazine (10 mg/kg) once. The rats were decapitated 24 hr after the final injection of syrosingopine or reserpine and 70 min after tetrabenazine.

*Significant difference from untreated ($p<0.05$).

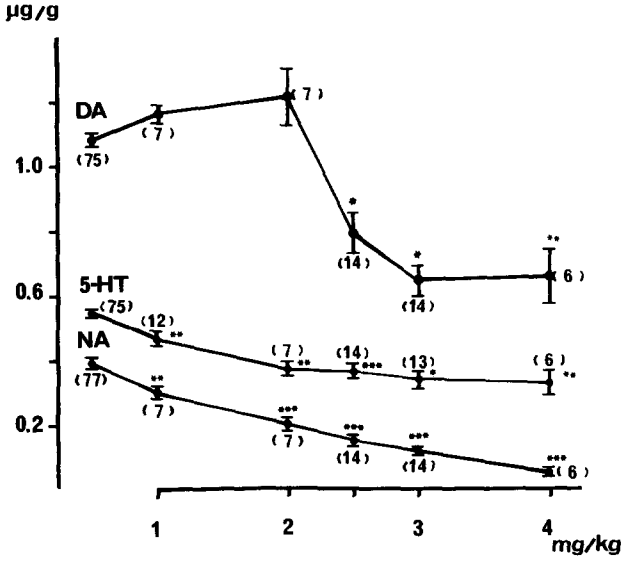

FIG. 3. Dose-response of brain amine levels to syrosingopine administered repeatedly in different 5 doses of 1, 2, 2.5, 3 and 4 mg/kg for 4 days. Further explanations as in Fig. 1.

induced depletion of brain amine as shown previously in Table 1. Syrosingopine (2 or 5 mg/kg) or reserpine (2 mg/kg) was administered daily for 2 days and tetrabenazine (10 mg/kg) once 10 min before morphine (5 or 10 mg/kg).

As seen in Fig. 5, morphine brought about marked increases of threshold voltages to vocalization, which were maximal 60 min and lasted more than 90 or 150 min after the injection according to the doses administered. Reserpine and tetrabenazine reduced this analgesic effect of morphine. Syrosingopine did not affect the morphine analgesia at a dose of 2 mg/kg, while it reduced the analgesic effect of morphine to a certain degree at a dose of 5 mg/kg.

DISCUSSION

From a number of studies, it is well documented that rauwolfia alkaloids, especially reserpine, induce a depletion of endogenous monoamines in various tissues. Reserpine releases intraneuronal amines as an inactivated form by disturbing their storage mechanisms and reduces uptake of amines into their storage sites, thereby causing the depletion of endogenous amines and less active amines available to the receptor sites in both central and peripheral adrenergic nerve terminals [12, 19, 20].

Garattini *et al.* [10] reported that the depleting effect of syrosingopine on rat brain 5-HT was about one tenth that of reserpine, and Orlans *et al.* [26] that syrosingopine was less effective in depleting brain stem NE and 5-HT than reserpine though both alkaloids were equipotent in depleting cardiac NE in rabbits and dogs. Similar results were demonstrated by Leroy [21] and Phm-Huu-Chanh *et al.* [27] in mice and dogs. Ishizaki [18] proposed that syrosingopine induced a slight increase of epinephrine and no alteration of NE level in the brain stem, in contrast to a marked reduction of both amines elicited by reserpine, and that the depleting effect of syrosingopine on cardiac NE was likewise weaker than that of reserpine. Liepman [22] *et al.* reported that the depleting effect of syrosingopine or rescinnamine on cardiac NE was one third of reserpine. These discrepancies in the reported results may be due to doses used and time elapsing between administration of alkaloid to decapitation.

It was reported later that syrosingopine, 2 mg/kg, induced a less marked depletion of 5-HT and a depletion of DA and NE, i.e., relatively selective depletion of brain amines, 4 hr after intravenous administration [38].

In the present study, DA, NE and 5-HT were simultaneously determined from individual animals and the responses to the alkaloids were determined dose- and time-dependently. From dose-related studies, it was demonstrated that all the 3 alkaloids reduced brain amines. However, reserpine was the most potent depletor of brain amines, rescinnamine the next and syrosingopine the weakest, while these 3 alkaloids were almost equipotent in depleting heart NE. Adrenal amines are less influenced by these alkaloids though reserpine was the most potent. Thus, syrosingopine, unlike reserpine, acts weakly on the central amines but acts potently on the peripheral amines.

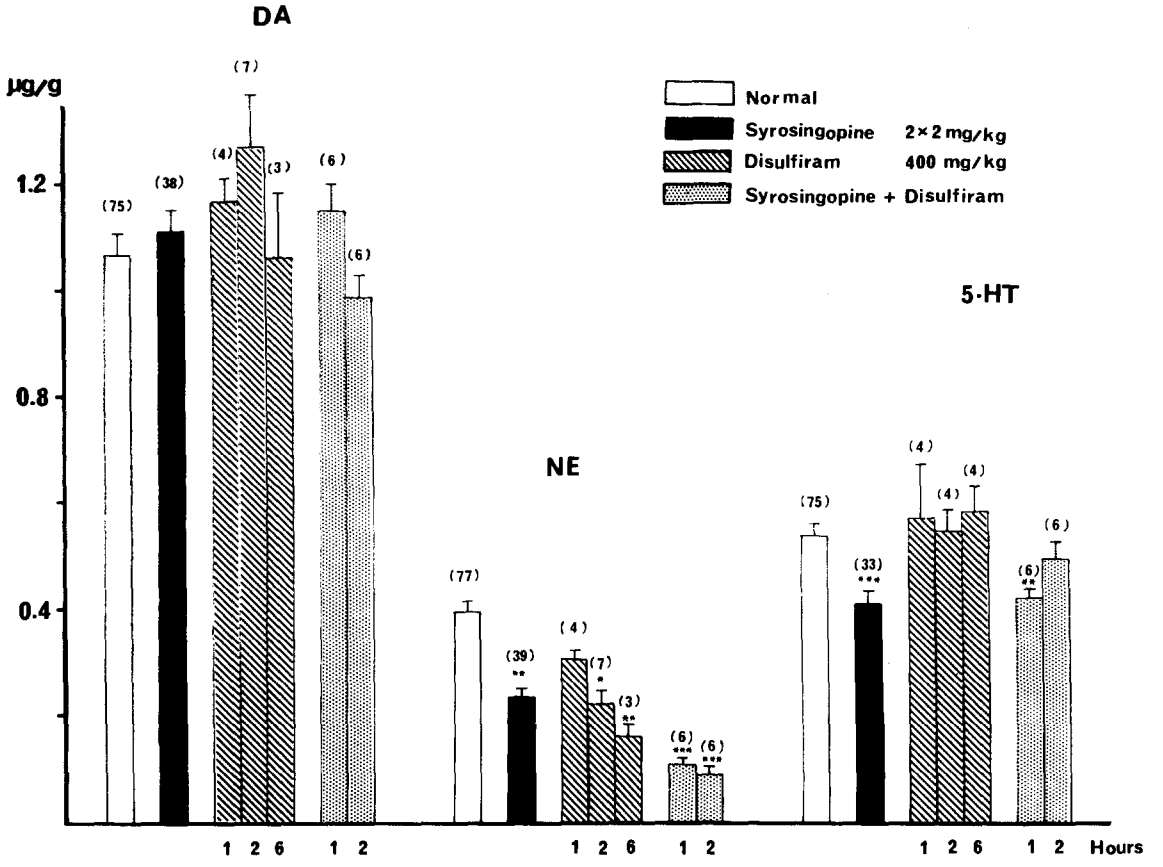

FIG. 4. Effect of combined use of syrosingopine with disulfiram on brain amine levels. Syrosingopine (2 mg/kg) was administered daily for 2 days and disulfiram (400 mg/kg) was injected alone or in combination with syrosingopine 1, 2 or 6 hr before decapitation.

From dose-response curves to reserpine and syrosingopine, reserpine (1 mg/kg) and syrosingopine (5 mg/kg) appear to be equally effective on brain amines. However, from time course studies, although both alkaloids produced similar alterations in brain NE and 5-HT levels, syrosingopine elicited only a weak and transient change in brain DA as compared with that caused by reserpine. This demonstrates that the levels of brain NE and 5-HT are lowered while that of brain DA is not altered 2 or 4 days after a single administration of syrosingopine, 5 mg/kg. The similar selective depletion of central stores of endogenous amines was also obtained by repeated treatments with smaller doses (1 or 2 mg/kg) of syrosingopine for 2 or 4 days.

Dopamine β-hydroxylase inhibitors such as disulfiram and fusaric acid are well documented to act selectively on the levels of brain amines. Disulfiram was reported to induce a slight increase of brain DA and a selective decrease of brain NE [13,15], and fusaric acid no change of brain DA and decreases of brain NE and 5-HT [16,24]. After the combined use of syrosingopine with disulfiram or with fusaric acid, a selective decrease of brain NE was potentiated whereas the alterations in the levels of brain DA and 5-HT were diminished.

Within recent years, numerous studies have suggested that brain monoamines may be involved in mediating some of the central nervous system effects of morphine. However, the question as to which of the amines play the most important role in the antinociceptive action of morphine remains to be elucidated. The analgesic effect of morphine has been proposed to be antagonized by rauwolfia alkaloids such as reserpine and tetrabenazine, a brain amines depletor [29, 30, 34]. Medakovic and Banic [23] have suggested that the inhibitory action of reserpine on the morphine analgesia is due to the releasing action of brain 5-HT since α-methyl-m-tyrosine which releases NE from brain stores without an appreciably depleting brain 5-HT stores antagonized the effect of morphine in mice. The reduced effect of morphine after reserpine-pretreatment could be restored by intraventricular injection of 5-HT but not by NE or DA [32]. On the contrary, it has been later demonstrated that the changes of brain NE plays a more important role than that of 5-HT in the antagonism [35]. Takagi, *et al.* [37] have lately reported that the treatment with dopa in tetrabenazine-treated mice recovered considerably the morphine analgesia as well as DA and NE contents in the brain, suggesting the involvement of brain catecholamines in the morphine analgesia. In the present study, the morphine analgesia was antagonized by reserpine and tetrabenazine but not by syrosingopine. This antagonism was most closely related to brain DA contents since reserpine and tetrabenazine depleted all brain monoamines whereas syrosingopine (2 mg/kg for 2 days) did not affect DA content with depletions of NE and 5-HT contents. In fact, syrosingopine induced a reduction in the analgesic

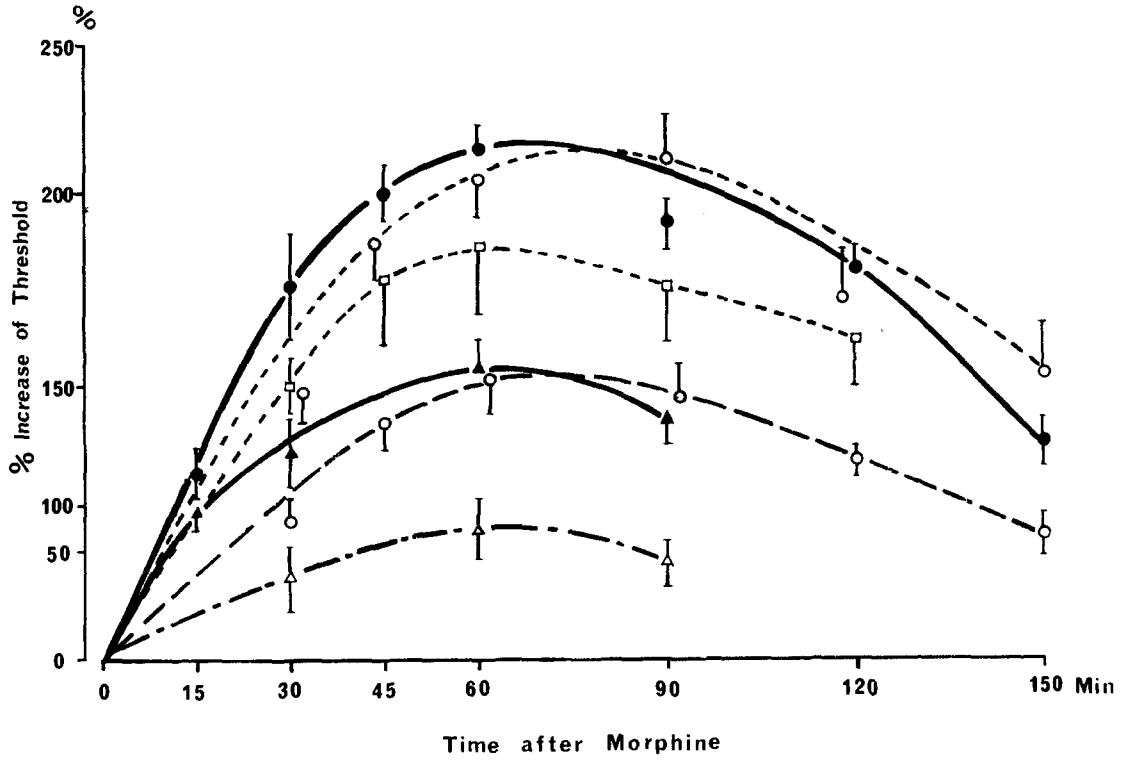

FIG. 5. Influences of syrosingopine, reserpine or tetrabenazine on alagesic effect of morphine. Each points show the means of percent increases of threshold for vocalization during electric stimulation and standard errors of the means. Syrosingopine (2 or 5 mg/kg) and reserpine (2 mg/kg) were administered twice 48 and 24 hr, and tetrabenazine once 10 min before morphine. ——●——; morphine 10 mg/kg (n = 52) ——▲——; morphine 5 mg/kg (n = 11) - - -○- - -; morphine 10 mg/kg after syrosingopine 2 mg/kg for 2 days (n = 6) - - -□- - -; morphine 10 mg/kg after syrosingopine 5 mg/kg for 2 days (n = 6) – –○– –; morphine 10 mg/kg after reserpine 2 mg/kg for 2 days (n = 18) – . –△– . –; morphine 5 mg/kg after tetrabenazine 10 mg/kg once (n = 10).

effect of morphine accompanied with a depletion of brain DA at a larger dose (5 mg/kg for 2 days). This result suggests a possible relation between DA concentrations and analgesic activity, and is compatible with previous findings that the analgesic action of morphine is potentiated by sodium diethyldithiocarbamate, an inhibitor of DA β-hydroxylase which produces a rise in DA concentration [39], and that a decrease in dopaminergic activity induced by 6-hydroxydopamine reduces the analgesic effect of morphine [7,25]. There have been some findings suggesting that DA might serve as inhibitory neurotransmitter in the central nervous system [3,9], and it appears to be general agreement that administration of morphine increases the turnover of DA in whole brain [8, 9, 31, 33]. As physiological and anatomical specifity in the central nervous system with regard to morphine analgesia has been very important subject to be elucidated, there have been some reports proposing that morphine also stimulates the turnover of DA in selected brain regions, such as the cortex [17,31], diencephalon [17,31], brain stem [31], corpus striatum [6, 11, 31, 33] and cerebellum [31]. Although future biochemical studies on localized brain regions after syrosingopine should be followed, the present result is thus favor of the hypothesis that DA participates in the mechanism of analgesic action of morphine [9, 33, 36].

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