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Some Observations on the Pharmacology of Mitragynine

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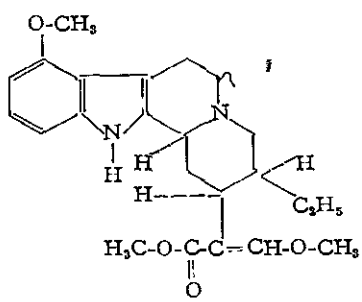
Abstract—Mitragynine (SK & F 12711), an indole alkaloid obtained from the tree *Mitragyna speciosa* possesses pain threshold elevating and antitussive properties in animals. Unlike the narcotic analgesics, this drug has little effect on gastric mobility, fails to produce excitement in cats, is not antagonized by nalorphine, has only weak respiratory depressant action in the anesthetized animal and is chemically unrelated to any known analgesic agent.

Introduction

Mitragynine (SK & F 12711), an indole alkaloid obtained from the tree, *Mitragyna speciosa*, was first isolated and named by Field (1) in 1921. She postulated the formula to be $C_{22}H_{31}O_5N$. More recently, in 1963 Joshi *et al.* (2) defined the structure of Mitragynine from a chemical study, and their findings were subsequently confirmed in X-ray crystallographic studies by Zacharias *et al.* in 1965 (3). Mitragynine has the molecular composition $C_{23}H_{30}N_2O_4$. The indole position is substituted with a methoxyl group, and is fused to a quinolizine ring system; this is substituted on adjacent carbon atoms with ethyl and methyl β-methoxyacrylyl groups. A *trans* relationship exists between the methoxy-



Mitragynine (SK & F 12711)

carbonyl and methoxy portions of the methyl β-methoxyacrylyl substituent of the quinolizine ring system.

The use of *Mitragyna speciosa* is well-documented in the folklore of the natives of Siam, Malaya, Borneo, the Philippine Islands and New Guinea. Manske (4) described its use in Siam, where the natives chew the leaves as a narcotic; in combination with the leaves of *M. parvifolia*, it enjoya an undeserved reputation as a cure for opium addiction. Grewel (5) discussed Mitragynine's bioactivity as a general protozoan poison, its effects on isolated tissues, and its actions on the autonomic nervous system of anesthetized animals. Grewel (6) also attempted to determine the effects of Mitragynine acetate on muscular and mental fatigue in a limited number of patients, but without success. Later, Thuan (7) described a single case of drug addiction with Mitragynine. In 1957, Douglas and Kiang of the University of Malaya supplied our laboratory with a crude mixture of the non-quaternary alkaloids from *M. speciosa*. This mixture was found to exhibit analgesic activity in animals, and it was decided to attempt to isolate the single pure constituent which might be principally responsible for elevating the pain threshold. Further investigation of this alkaloid seemed warranted, since the literature contains little information on the pharmacological action of the alkaloids of *M. speciosa*. Our preliminary studies indicated that the single pure alkaloid might be extremely potent, and that it might offer some advantage over existing analgesic drugs.

Methods

In this review, all doses of the compounds tested are expressed in terms of the free base. SK & F 12711 (1) was administered as either the hydrochloride salt, designated '-A', or the ethanedisulfonate salt, '-J'. To convert the free base dose to either salt form, the free base value should be multiplied by 1.10 or 1.25, respectively. The salt form of the other compounds used, and the appropriate conversion factors (for free base to salt form), are as follows: codeine as the phosphate salt (1.41), and dextropropoxyphene as the hydrochloride salt (1.11). SK & F 12711-A or -J was prepared as a weakly acidified aqueous solution of pH 4 to 5. All standard agents were freely soluble in water. Unless otherwise stipulated, the ED_{50} values were calculated by the method of Litchfield and Wilcoxon (8); these values indicate dose levels of the free base at which 50 per cent of the animals exhibit a significant pharmacological effect. In all of the experiments except the subacute toxicity studies, the Sprague-Dawley strain of rat was used.

Reaction to Nociceptive Stimulus

1) *D'Amour and Smith test.* Analgesia is defined as an elevation of pain

(1) This pure alkaloid was isolated from the leaves of the Malayan plant, *Mitragyna speciosa*, by Dr. Arnold H. Beckett, Chelsea College of Science and Technology, London.

threshold; it was determined by a modification of the D'Amour and Smith procedure (9). Fasted rats are restrained in cylindrical wire cages. The tails are blackened with Flo-master (1) quick-drying black ink to produce uniform light absorption, and the noxious stimulus is delivered by a 100 watt CDJ General Electric projection lamp (2,000 lumens, 120V), housed in a semi-polished aluminum box which acts as a reflector. A convex lens produces a focal point 3 mm. in diameter. The intensity of the light beam is regulated by a rheostat, calibrated so that various settings produce 220, 240, 300 or 350 mcal/cm²/sec., respectively. The rheostat is adjusted to give the desired average reaction time (usually 4-5 sec.). A timing mechanism works simultaneously with the light. The rat's tail is placed on a grooved block at the focal point of the light beam. The light is applied and then extinguished when the rat flicks its tail; automatically measuring the reaction time.

Animals are screened twice, with a 30 minute interval. The individual reaction times must fall within one second of each other, and the total range can be no more than 3 sec. Any animal whose reaction times do not meet these criteria is eliminated from the study. The cutoff value is approximately the average reaction time, plus two standard deviations. Any animal with a reaction time equal to or greater than the cutoff time is considered to exhibit analgesia.

Analgesia is calculated quantally, and provision for 'Analgetic' controls is made according to the following formula:

$$\frac{\%\ \text{experimental} - \%\ \text{control}}{100\ \% - \%\ \text{control}} \times 100$$

2) *Hardy, Wolff and Goodell test.* A modification of the Hardy, Wolff and Goodell (10) test described by De Sanctis *et al.* (11) employs trained dogs to measure analgesia. Briefly, the apparatus (2) consists primarily of a Dolorimeter or a control unit, and a projector with a projection lamp. The projector is connected to an electric timer which permits a clock to start and stop simultaneously with the operation of the projector. A Dermal Radiometer is used to measure skin temperature. The intensity of radiation, which is 250 mcal/sec/cm² for this test, is calibrated by means of a thermopile and galvanometer.

For a period of two weeks, dogs are trained to stand in a harness. The surface of the skin of the hind legs of each dog is clipped and blackened. Flo Master Ink is used in preference to India Ink of Vulcanol, since it is applied easily, dries rapidly, and provides a consistent absorption spectrum for at least 24 hours after application. To obtain precise measurements of the reaction threshold (withdrawal of leg), which is directly related to the initial skin temperature (which, in turn, may be subject to change under the influence of a drug), the skin temperature is taken by a Dermal Radiometer. Using the following equation,

(1) Esterbrook Pen Company, Camden, New Jersey, Transparent black ink, Special formula for felt tip pens. Stock #T-104.

(2) Equipment purchased from Williamson Development Company, Massachusetts.

it is possible to calculate the skin temperature level reached following irradiation of the skin at the time of pain (reaction to stimulus).

$$Tpt = TF \pm QK\,t$$

Tpt = skin temperature at pain threshold (°C).
TF = normal skin temperature before irradiation (°C).
Q = intensity of radiation (mcal/sec/cm²).
K = constant.
t = time of exposure in seconds.

In the present studies, two control thresholds were determined, one on each leg. These determinations were repeated one hour later. After administration of the compound, determinations were made at 1, 2, 3, 4, and 22 hours post drug. Hardy *et al.* found that exposure beyond 67°C caused tissue damage; therefore, no exposure was carried beyond this temperature. Using Hardy's method, it was possible to determine the number of animals in a group which demonstrated analgesia; each dog was his own control, and a cutoff value could be calculated for each animal, since a minimum of twenty control readings was obtained for each dog before drug testing. In addition, the method of Finney's Error Regression Analysis for a Discriminant (12), in which the duration of exposure to radiant heat alone is examined, determines the statistical significance of the drug-treated animals as a group (at least 5 animals per group) with reference to the presence or absence of 'analgesia'.

3) *Randall and Selitto test.* A pressure on inflammed rat paws, according to the technique of Randall and Selitto (13), was utilized. The inflammation was induced by the subaponeurotic injection of 0.1 ml of 20 % dried Brewer's yeast suspension into the plantar region of the hind foot. Pain stimulation in the inflammed paw was induced by exerting pressure with a semi-pointed plastic projection attached to the barrel of a vertically mounted 10 ml hypodermic syringe. The pain threshold was measured by the amount of pressure in mmHg necessary to induce a flight reaction in the rat (100-110 grams). Oral Mitragynine was administered immediately after the yeast was injected, and the pain threshold was measured 1 and 2 hours after drug treatment. Analgesia was calculated by the same analysis used in the D'Amour and Smith Method.

4) *Hot plate test.* The method employed for analgesic effect was that described by Eddy (14). This procedure determines the reaction of mice (CF$_1$, 18-25 grams) dropped onto a hot plate maintained at 54.5 ± 1.5°C. The reaction was observed as a lifting or kicking of the hind leg, dancing about the restraining cylinder, or attempting to jump out of the cylinder. Reaction time for each mouse was determined twice before drug administration, 10, 20, 30, 45 and 60 minutes after drug administration, and then every 30 minutes until the reaction time returned to control levels.

The criterion of analgesic effect in a single animal was the difference between the calculated reaction time area for the first 60 minutes after injection, and

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Antitussive Test

The test employed for antitussive activity was that of Tedeschi *et al.* (15). The cough reflex in unanesthetized dogs was produced by using an electromagnet to attract an iron slug which had been permanently suspended in the trachea on the tracheal wall. In the test procedure, the energized electromagnet was placed at the dog' throat over the iron slug for a 3 second period, followed by a rest period of 30 seconds. The number of evoked coughs was recorded during this 33 second interval. This procedure was repeated 5 times to establish an average control value for each dog. After oral or subcutaneous drug treatment, the animals were tested at hourly intervals for 5 hours, and then at 24 hours. The dose of drug which produced a 50 % depression in cough episodes (ED_{50}) was calculated from the regression obtained by plotting the average per cent suppression in the frequency of cough episodes against log dose (12). At least three dose levels were studied with each drug, and a minimum of 5 dogs was tested at each dose level.

Charcoal Meal Test

Groups of 10 rats (180-220 grams) were deprived of food, but not water, for a period of 20-24 hours before testing. The rats were treated orally or intraperitoneally with drug at a pre-determined time prior to the oral administration of 0.5 ml/100 grams of 7.5 % charcoal (Norite), suspended in 0.375 % tragacanth gel. The pretreatment time for the drug was chosen so that the peak activity of the drug coincided with the administration of charcoal. Thirty minutes after charcoal administration, the rats were sacrified by being placed in an ether chamber, and the gastro-intestinal tracts were swiftly removed. The pylorus was used as the point of origin, and the distance the charcoal traveled through the intestine was measured and recorded. Controls were run simultaneously with the treated groups. The method of Litchfield and Wilcoxon (8) was used to calculate the ED_{50}'s for the standard agents.

Nalorphine Antagonism Tests

Mitragynine was administered intraperitoneally to cats in dose levels of 18.4 mg/kg. When peak overt effects appeared, a dose of 10 mg/kg of nalorphine was given intraperitoneally to determine if nalorphine would antagonize the overt effects. In other experiments, two groups of nine rats were pretreated orally with an analgesic dose (AD_{78}) of Mitragynine. One group received 3.5 mg/kg of nalorphine intraperitoneally immediately after receiving Mitragynine. All the animals were tested for analgesia 45 minutes after drug treatment, using the previously described D'Amour and Smith test.

Cardiovascular Tests

Cardiovascular studies were carried out in cats anesthetized with chloralose or in dogs anesthetized with pentobarbital, ether chloralose, or urethane. Mean carotid blood pressure was recorded on a smoked kymograph paper, using a mercury manometer. The integrity of the autonomic nervous system was monitored by the injection of specific test agents. Respiration (tidal volume and rate) was measured by cannulating the trachea with a glass "T" tube connected to an Anderson glass inspirometer. A flutter valve was placed over the remaining arm of the "T" tube. The system was calibrated by clamping off the tube leading from the inspirometer to the trachea, and withdrawing a given volume of air with a 50 ml syringe. The net change in height of the recording lever (inm) then corresponded to the given volume of air withdrawn.

Dose Range Tests

Graded doses of Mitragynine were administered orally, intravenously, or parenterally to mice, rats, cats, dogs or monkeys. The animals were continuously observed for overt effects over a period of 4-6 hour intervals after treatment and again at 24 hour intervals, for 5 days.

Animal Toxicity

1) *Acute toxicity studies.* Tests were carried out in groups of ten male rats (150-170 grams), which received single oral doses of 525 and 807 mg/kg of Mitragynine. Observations were made for six hours following drug administration, and daily for seven days.

2) *Subacute administration.* A group of 12 rats received oral doses of 8 mg/kg/day for five consecutive days. Observations were made for six hours after dosing on each day of the test. Two dogs received oral doses of 16.1 mg/kg/day of Mitragynine for five days, and 32.2 mg/kg/day for two additional days.

3) *Subacute toxicity studies.* Two groups of eight and ten male rats and eight and ten female (Charles River Strain) rats received oral doses of 5 or 50 mg/kg/day (free base) of Mitragynine, five days a week for six weeks. The daily dosages were administered via stomach tube in suspension with 0.5 % tragacanth gel.

Twelve purebred Beagle dogs were divided into three groups; each consisted of two males and two females. Group I animals were untreated, and served as controls. Group II animals received 5 mg/kg/day of Mitragynine orally, six days a week for eight weeks. The animals in Group III were started on 20 mg/kg/day of Mitragynine six days a week for 3 weeks. They received 40 mg/kg/day from day 22 to day 50, and the drug was withheld from day 51 through day 92.

On day 93, a dose of 40 mg/kg/day of Mitragynine was administered for an additional 10 days and the animals were sacrificed and autopsied; two on day 103, one on day 117 and the last dog on day 144 of the test period.

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SK & F 12711-A
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Codeine
d-Propoxyphene

Miscellaneous Tests

Mitragynine was tested for its effect on the isolated rabbit ileum in accordance with the method of Magnus (16).

Mitragynine was tested for physical dependence in the morphine dependent monkey by Drs. Deneau and Seevers (17) at Ann Arbor, Michigan.

Conditioned avoidance response activity was tested with the method of Cook *et al.* (18); groups of 10 rats each received oral doses of 50 mg/kg of Mitragynine, and were tested at 45, 90, 180 and 240 minutes after treatment. Groups of 10 rats also received intraperitoneal doses of 20 or 40 mg/kg, and were tested at 30, 60, 90, 120 and 210 minutes after treatment for an effect on the conditioned escape response.

Hypoglycemic tests were carried out in fasted guinea pigs treated with Mitragynine. Forty-five minutes after the administration of oral doses of 30 mg/kg, the animals were sacrificed by exsaguination. Blood was collected, and blood glucose was determined with the Technicon Auto Analyzer by the method described by Hoffman (19).

Results

Analgesia

The analgesic activity of Mitragynine was evaluated in the fasted rat, using modifications by the D'Amour-Smith and the Randall and Selitto methods. The results obtained using these test procedures are summarized in Table I. From the data presented, Mitragynine appears to be slightly less active orally than codeine in both tests. When given at a subcutaneous dose of 31 mg/kg, Mitragynine produced analgesia in only 33 % of the animals in the tail flick test, compared to an ED_{50} of 8.0 mg/kg (6.0–11.2 mg/kg) for subcutaneous codeine. Intraperitoneally, Mitragynine has an ED_{50} of 14.4 mg/kg (7.6–27.3 mg/kg); this is in the range of potency achieved by the oral route.

TABLE I

Oral ED_{50}'s (mg/kg) for analgesia of compounds tested in the rat

Drug	Method	
	D-Amour and Smith ED_{50} (95 % fiducial limits)	Randall and Selitto ED_{50} (95 % fiducial limits)
SK & F 12711-A	20.2 (11.8–34.4)	18.4 (5.7–59.5)
SK & F 12711-J	17.8 (9.1–34.5)	16.8 (8.1–22.7)
Codeine	10.3 (5.2–20.6)	7.5 (4.5–12.6)
d-Propoxyphene	9.0 (4.5–18)	27.9 (18–42)

Mitragynine exhibited good pain threshold elevating properties in the dog. The data summarized in Table II show that the analgesic activity exhibited by Mitragynine was comparable to that produced by codeine and dextropropoxyphene. For purposes of comparison, phenacetin, a mild analgesic is inactive in this test at an oral dose of 200 mg/kg. It is interesting to note that, while codeine caused emesis and dyspnea in these animals, Mitragynine was devoid of these particular properties.

TABLE II

Comparative pain threshold elevating properties of several drugs in trained unanesthetized dogs [a]

Drugs	Oral dose mg/kg	No. of animals *exhibiting analgesia* no. of animals tested	Major side effects
SK & F 12711-J	2.0	2/5	anorexia, shivering, slight decrease in spontaneous *motor activity.*
	4.0	6/11	shivering, slight restlessness, quiet sedate.
	8.0	3/5	
	12.0	2/2	
	16.0	2/2	slight decrease in spontaneous *motor activity*
	24.0	5/5	slight shivering, slight ataxia, slight *restlessness.*
Codeine	1.8	3/5	no side effects.
	3.5	4/12	emesis.
	7.1	7/13	emesis, anorexia.
	10.6	5/7	emesis, dyspnea.
	14.1	2/6	emesis, anorexia, increased motor activity.
Dextro-Propoxyphene	2.3	2/5	slight excitation.
	4.5	4/5	slight restlessness.
	9.0	4/5	slight ataxia, slight to moderate *depression.*
	13.5	14/15	slight to moderate ataxia, slight to moderate depression.

The oral and subcutaneous analgesic activities of Mitragynine and codeine were evaluated in mice by the Hot Plate Method of Eddy. The results summarized in Table III, again demonstrate that Mitragynine is more potent when given orally than when administered subcutaneously.

Effect of SK & F 12711-

Drug
SK & F 12711-A
Codeine
SK & F 12711-A
Codeine

Antitussive ac

SK & F 12711	
Dose mg/kg	Average r
0.9	
2.3	
4.5	
9.0	
SK & F 12711-	
0.9	
2.3	
4.6	
9.2	
Codeine PO	
1	
2	
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8	
15	

* Values represent means of th
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TABLE III

Effect of SK & F 12711-A and codeine on pain threshold of mice measured by the hot plate method

Drug	Dose mg/kg	Route	% Analgesia
SK & F 12711-A	92	p.o.	100 %
Codeine	35.2	p.o.	90 %
SK & F 12711-A	92	s.c.	0 %
Codeine	21.3	s.c.	70 %

TABLE IV

Antitussive activity in the trained unanesthetized dog

SK & F 12711-J *ED_{50} = 1.8 mg/kg (1.0–2.6 mg/kg)

Dose mg/kg	Average maximum inhibition %	Observation
0.9	36	No side effects
2.3	51	No side effects
4.5	70	1/3 defecation
9.0	100	2/3 slight stimulation and defecation, 1/3 salivation.

SK & F 12711-A **ED_{50} = 2.3 mg/kg (1.3–3.3 mg/kg)

0.9	27	No side effects
2.3	57	1/5 restlessness
4.6	65	2/5 restlessness at 3 hr.
9.2	92	2/5 slight ataxia, 2/5 slight apprehension

Codeine PO_4-ED_{50} = 4.3 mg/kg (2.1–9.1 mg/kg)

1	25	No side effects
2	36	2/6 emesis
4	48	2/6 emesis
8	56	8/8 emesis, relaxed nicitating membranes.
15	82	4/5 emesis, hypotonia, salivation, bradypnea, tense, decrease in motor activity, 1/5 severe retching.

* Values represent means of three animals per dose.
** Values represent means of five animals per dose.

Antitussive Activity

In the trained unanesthetized dog, Mitragynine and codeine were approximately equipotent in suppressing the cough reflex. As shown in Table IV, the oral ED_{50} for Mitragynine HCl was 2.3 mg/kg, and that for Mitragynine ethane disulfonate was 1.8 mg/kg. In this test, codeine had an ED_{50} of 3.5 mg/kg. It should be noted that, in this series of animals, there was no emesis with Mitragynine at the dose levels employed. On the other hand, it is appropriate to mention that the oral activity (ED_{50} value) of codeine must be accepted with some reservation, since codeine produced considerable emesis, especially at the higher dose levels.

Effects on the Gastrointestinal System

As shown in Table V, Mitragynine failed to inhibit the gastrointestinal propulsion of a charcoal meal in rats after intraperitoneal doses of 36.8 mg/kg. Oral doses of 55.2 mg/kg produced inhibition of only 18 %. Codeine has an ED_{50} (that dose which inhibits propulsion by 50 %) of 25.2 mg/kg intraperitoneally, and orally it appears to be slightly more active than Mitragynine in inhibiting intestinal motility. Of the compounds tested, morphine produced the greatest inhibition of the passage of charcoal meal.

TABLE V

The comparative effect of drugs on charcoal meal passage in rats

Drug	Dose (mg/kg) and Route	% Inhibition
	i.p.	
Codeine	42.6	70
ED_{50} = 25.2 mg/kg	21.3	46
(14.8 to 42.9 mg/kg)	10 6	16
	5.3	7
Morphine hydrochloride	7.6	73
ED_{50} = 3.53 mg/kg	3.8	50
(1.43 to 7.60 mg/kg)	1.9	34
SK & F 12711-A	36.8	8
	36.8	9
	p.o.	
Codeine	42.6	35
	21.3	22
SK & F 12711-A	55.2	14
	55.2	18

Mitragynine was found to ex
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Nalorphine Antagonism Studi

1) *Cat.* Intraperitoneal dose
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2) *Rat.* The analgesic actio
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Effect of nalorphine o

Drug, Dose (mg/kg, Route)	T Pe
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SK & F 12711-A 32.2 mg/kg p.o.	

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d Route	% Inhibition
	70
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	16
	7
	73
	50
	34
	8
	9
	35
	22
	14
	18

Mitragynine was found to exhibit anticholinergic activity in normal isolated rabbit and guinea pig ileum preparations. In this regard, however, Mitragynine was far less potent than atropine. From these studies, it would seem that the anticholinergic action of Mitragynine would probably be negligible at analgesic dose levels.

Nalorphine Antagonism Studies.

1) *Cat.* Intraperitoneal doses of 18.4 to 46 mg/kg of Mitragynine produced subtle behavioral changes in cats; these were characterized by very mild stimulation, mydriasis, and restlessness.

Nalorphine was injected at a dose of 10 mg/kg intraperitoneally, one hour after doses of 18.4 mg/kg of Mitragynine had been injected. It was difficult to observe any changes in the behavior of the cat following the nalorphine injection, although the animal appeared somewhat less restless for 2 or 3 hours following nalorphine treatment. In contrast, both codeine and morphine produced marked stimulation in cats following intraperitoneal doses of 2.5 and 30 mg/kg, respectively. Intraperitoneal doses of 10 mg/kg of nalorphine given one hour after the codeine or morphine injections caused the behavior of the cats to return to an almost normal pattern. Another difference was observed in this test; morphine and codeine caused some slowing of respiratory rate, but Mitragynine did not produce any change. In cats pretreated with morphine or codeine, the injection of nalorphine produced a marked increase in respiratory rate, but did not influence the respiratory rate in the Mitragynine-treated cat. In cats, Mitragynine caused marked mysriasis, which was antagonized by the injection of nalorphine. The mydriasis produced by morphine and codeine was also antagonized by the injection of nalorphine.

2) *Rat.* The analgesic action produced by SK & F 12711-A in the rat was not antagonized by the simultaneous intraperitoneal injection of nalorphine. Using the D'Amour and Smith technique, one group of ten rats received an oral dose of 32.2 mg/kg of Mitragynine, while a second group received 32.2

TABLE VI

Effect of nalorphine on pain elevation produced by mitragynine

Drug, Dose (mg/kg, Route)	Time of Peak Effect (Min.)	No. Analgesia / No. Tested	% Analgesia
SK & F 12711-A 32.2 mg/kg p.o. plus Nalorphine 3.5 mg/kg i.p.	45	7/9	78
SK & F 12711-A 32.2 mg/kg p.o.	45	7/9	78

mg/kg of SK & F 12711-A orally, and 3.5 mg/kg of nalorphine intraperitoneally. The results are presented in Table VI.

Cardiovascular and Respiratory Effects.

In experiments carried out on dogs anesthetized with pentobarbital, SK & F 12711-A produced minimal alterations in mean arterial blood pressure (≤ 20 mm Hg) after acute intravenous doses of from 0.092 to 9.2 mg/kg, or a cumulative dose of 18.5 mg/kg. In one experiment, codeine had no significant effect on the blood pressure after doses up to 3.5 mg/kg. However, in a second experiment, a dose of 0.7 mg/kg of codeine resulted in a profound sustained hypotension. No alterations in the responses to standard agents such as epinephrine, norepinephrine, dimethylphenylpiperazinium, or furfuryl trimethyl iodide were observed when these agents were given after SK & F 12711-A. There was no alteration in the depressor response produced by histamine, or the stimulation of the peripheral stem of the vagus nerve.

Chloralose was used as the anesthetic in 3 separate experiments in cats. SK & F 12711-A produced weak hypotension after an acute intravenous dose of 0.46 or 0.92 mg/kg in one cat, and after 4.6 mg/kg in another animal (9.2 mg/kg was lethal to this animal). In the third cat, doses of 0.46 mg/kg to 2.3 mg/kg produced a transient lowering of mean arterial blood pressure. In this animal, death due to respiratory failure was observed after a dose of 4.6 mg/kg.

The observations in our experiments indicate that codeine is more active than SK & F 12711-A in depressing respiration following intravenous injection in anesthetized dogs. In the nembutalized animal both compounds initially increased respiratory rate. However, following this increase, respiratory depression was more frequent and more marked after codeine than after SK & F 12711-A. Codeine also significantly reduced tidal volume and respiratory rate in one animal anesthetized with ether-chloralose. This depressing action of codeine was very slight in the dog anesthetized with urethane. In the ether-chloralose and urethane treated animals, SK & F 12711-A increased tidal volume.

Dose Range Studies.

SK & F 12711-A produced central nervous system depression, characterized by decreased spontaneous motor activity, at doses of 46 to 230 mg/kg orally. In addition, mydriasis, increased pain threshold, bradypnea, and hypothermia were noted. No evidence of toxicity (convulsions or tremors) was observed after doses as high as 920 mg/kg in the mouse. Codeine produced toxicity (gasping, clonic convulsions, death) after doses of 176 mg/kg orally in the mouse and, in addition, differed from SK & F 12711-A by producing hypersensitivity at the higher dose levels. In the rat, oral doses of Mitragynine as high as 807 mg/kg failed to produce lethality. Only slight depression of spontaneous motor activity, ptosis, and ataxia were observed at this dose level in the rat.

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31.8 mg/kg intravenously.

Side effects

Drug	Dose mg/kg (i.p.)
SK & F 12711-A	9.2
	18.4
	46.0
Codeine	1.7–3.5
	7.1
	14.1
Morphine SO_4	1.9
	3.8
Dextro-propoxyphene	9.0
	18.0
	27.0
	36.0
	45.0

As shown in Table VII,
peritoneally, produced in c
ploratory behavior. The de
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With the latter compounds,

In the dog, oral doses of 8 to 80 mg/kg of Mitragynine failed to produce gross observable side effects; an intravenous dose of 4.6 mg/kg of Mitragynine was also without effect. An intravenous dose of 9.2 mg/kg produced some respiratory slowing, ataxia and defecation. Severe side effects, such as clonic convulsions, respiratory depression, panting and prostration, occurred following 31.8 mg/kg intravenously.

TABLE VII

Side effects produced by analgesic drugs in cats

Drug	Dose mg/kg (i.p.)	Observations
SK & F 12711-A	9.2	2/2 slight mydriasis lasting longer than 2 hours.
	18.4	2/2 mydriasis; 1/2 stimulation; 1/2 inquisitive; 1/2 head movements; 1/2 hypersensitive to touch; 1/2 slight apprehension; 1/2 rubbing cage; 1/2 quiet.
	46.0	2/2 restless, inquisitive (not stimulated), head movements.
Codeine	1.7–3.5	2/2 mydriasis; 1/2 defecation, salivation, marked stimulation, cowering, hissing, disorientation, body taut.
	7.1	2/2 mydriasis, salivation, slight stimulation, cowering, hissing.
	14.1	2/2 mydriasis; 1/2 salivation, cowering, hissing.
Morphine SO_4	1.9	2/2 mydriasis, emesis, defecation, tense, disoriented, very excited, apprehensive, hypersensitive to touch.
	3.8	2/2 mydriasis, excited, relaxed nicitating membrane.
Dextro-propoxyphene	9.0	1/1 mydriasis, convulsions, salivation, dyspnea, tremors. Overnight—mod. decreased activity, ataxia.
	18.0	2/2 mydriasis, convulsions, salivation, dyspnea, prostration, 1/2 disoriented, death within 1 hour.
	27.0	2/2 mydriasis, stimulation, cowering, hissing, convulsions, salivation, 1/2 disoriented, 1/2 ataxia, 1/2 dyspnea, 1/2 prostration, 1/2 unilateral foreleg clonus, 1/2 head tremors.
	36.0	2/2 mydriasis, disorientation, stimulation, body jerks.
	45.0	1/1 mydriasis, alert.

As shown in Table VII, SK & F 12711-A, in doses of 18.4 mg/kg intraperitoneally, produced in cats mydriasis, stimulation, and an increase in exploratory behavior. The degree of mydriasis was marked, but the stimulatory effects appeared qualitatively different than the effects after codeine or morphine. With the latter compounds, stimulation was accompanied by a profound hyper-

sensitivity to external stimuli—a 'fear complex', which manifested itself by causing the animal to cower in the corner or move jerkily to escape the cage, or by producing a condition simulating rage. These effects were not consistent in any group of animals—especially the rage effect, which occurred in approximately 50 % of the animals at appropriate dose levels. Unfortunately, the low solubility of SK & F 12711-A limited the dosage level, so it was not possible to determine the toxic dose levels or the qualitative similarities which might occur with high doses of Mitragynine as compared to codeine or morphine. As shown in Table VII, an intraperitoneal dose of 46 mg/kg of Mitragynine produced only slight mydriasis.

It is interesting to note, however, that differences were obvious at the 18.4 mg/kg dose levels. As stated previously, Mitragynine caused mydriasis and mild stimulation. Codeine caused mydriasis, stimulation, salivation, and a fear complex. Morphine sulfate produced severe side effects at 7.5 mg/kg; these included marked excitement, disorientation, mydriasis, emesis, etc. Dextropropoxyphene hydrochloride was toxic at 20 mg/kg intraperitoneally, causing mydriasis, disorientation, stimulation, and convulsive seizures. In preliminary testing (unpublished observation), using a modification of the Hardy Wolff and Goodell procedure (10) in cats, Mitragynine elevated pain threshold at oral doses of 8 mg/kg, without producing side effects. Dextropropoxyphene produced analgesia at 11 mg/kg, but caused salivation and mydriasis.

SK & F 12711-A failed to produce overt biological activity in monkeys following subcutaneous doses ranging from 23 to 69 mg/kg. One monkey treated with 25.7 mg/kg subcutaneously, and injected 24 hours later with 23 mg/kg intramuscularly, also failed to show overt activity. In three monkeys, an intravenous dose of 9.2 mg/kg of SK & F 12711-A produced ataxia, slight opisthotonus, slow abdominal respiration and clonic convulsions. All animals recovered after 5–30 minutes. A dose of 4.6 mg/kg failed to cause side effects. A single oral dose of 46 mg/kg failed to alter the usual hostile behavior in a Rhesus monkey.

Animal Toxicity.

1) *Acute.* Single oral doses as high as 806 mg/kg failed to produce toxicity in rats. Oral administration of SK & F 12711-J at a dose of 8 mg/kg/day for five days produced only diarrhea in 3/12 animals. No other side effects were observed. Two dogs which received oral doses of 16 mg/kg/day for five days, and 32 mg/kg/day for two additional days, failed to exhibit side effects.

2) *Subacute — rats.* The administration of 5 or 50 mg/kg/day of SK & F 12711-J, five days a week for six weeks, failed to produce side effects in any of the rats. There were some slight weight changes; the body weight of the low dose males averaged slightly less than the control males, and the average net gain of body weight of the females receiving the high dose was slightly more than the control females. Food consumption in all of the treated animals was similar to that of the controls. A statistically significant decrease in the

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3) *Subacute — dog*
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Miscellaneous.

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Discussion

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actual weight of the livers of the low dose group was observed, whereas relative organ weight data showed a statistically significant decrease in liver weights of the high dose males, and kidney weights of the low dose females. No hematological, urinalytical or histopathological changes which could be attributed to drug administration were observed in any of the animals.

3) *Subacute - – dogs.* Significant clinical pathological findings were limited to the dogs which initially received 20 mg/kg/day of SK & F 12711-J, six days a week for three weeks. Since no adverse side effects were observed at this dose level after three weeks of drug treatment, the dose was increased to 40 mg/kg/day, six days a week from day 22 to day 50. Then the clinical findings were characterized by leukopenia, granulocytopenia, lymphocytosis, monocytosis, and atypical and immature lymphocytes. These changes were reversed after the drug had been withheld from days 50 to 92 but recurred when dosing with the drug was resumed. In addition, costal bone marrow specimens from the high dose dogs revealed moderately severe granulocytic hyperplasia in the male, but less in the female dogs. Lymph nodes were also hyperplastic in 3/4 of these animals. Finally, diffuse increased sinusoidal cellularity was observed in the livers in 3/4 of the high dose dogs. These findings were considered to be related to drug administration. Negative findings were found in the dogs receiving oral doses of 5 mg/kg/day of Mitragynine for three weeks.

Miscellaneous.

Mitragynine failed to inhibit the conditioned avoidance response in rats. The compound failed to affect the blood sugar in fasted guinea-pigs, whereas, in concomitant tests, codeine produced slight hypoglycemic activity.

Discussion

In our search for potent, yet safe analgesics, possessing a profile of qualities which are superior to those of existing agents, Mitragynine exhibited activity in animals which seemed of potential usefulness in man. The compound, chemically unrelated to any of the known analgesic agents, appeared qualitatively different from the narcotic analgesics, and further pharmacological evaluation of its properties appeared warranted.

Mitragynine exhibited analgesic activity in at least three species of animals. It differs from other analgesics by elevating the pain threshold in the rat, mouse and the dog with only minimal side effects. There was no indication of Straub tail in the rodent, nor was there evidence of the severe emesis, hyperexcitability or severe respiratory depression seen with narcotic analgesics in larger animals, such as the cat or the dog. Mitragynine has an oral-intraperitoneal ratio of approximately 1 : 1 in producing analgesia in the rat, whereas it is practically inactive following the subcutaneous route. It is difficult to explain the difference

in Mitragynine's effectiveness in producing analgesia following various routes of administration—especially the differences between the intraperitoneal and subcutaneous routes of administration. These differences may be related to the presently unresolved physical properties of the compound. Mitragynine is sparingly soluble in acidified aqueous solution of pH 4 to 5. Elevating the pH above 6 causes an uninjectable gelatinous mass to form. Thus, absorption by the oral route may be facilitated by stomach acidity. It is also possible that the active analgesic moiety may be due to a metabolite of Mitragynine, and that oral administration facilitates the transformation of Mitragynine to an active moiety by involving the most optimal processes. Unfortunately, our limited exploration of this problem did not provide further information on the underlying processes that account for the differential in animal sensitivity to the different routes of administration. Further exploration of the various metabolites of Mitragynine and its closely related congeners may provide a better insight to this perplexing problem.

It is interesting to mention here that subcutaneous doses of Mitragynine ranging from 0.46 to 22 mg/kg did not suppress abstinence signs in monkeys physically dependent on morphine (20). Subcutaneously, therefore, Mitragynine does not appear to support morphine-induced addiction, although there is no evidence that Mitragynine is active in the monkey by this route of administration. The analgesia produced by narcotic drugs such as morphine can be antagonized by nalorphine. In preliminary studies, Mitragynine's analgesic action was not antagonized by nalorphine in the rat. In addition, Mitragynine appears to be qualitatively different than morphine or codeine in producing behavioral changes in the cat. With morphine and codeine, cats exhibit markedly dilated pupils, slow respiratory rate, stimulation, cowering, hissing, and many effects indicative of a rage complex. Mitragynine is qualitatively different from the narcotic analgesics in that cats exhibit only a weak stimulation that is more akin to restlessness. There was no evidence of disorientation nor any discernible influence on respiration. When nalorphine was given after Mitragynine, there was no evidence of the panting or increased respiration that was noted with the narcotic analgesics. These observations are in agreement with the studies carried out in the pentobarbitalized or urethane anesthetized dogs, in which Mitragynine was approximately 1/10th as effective as codeine in depressing the tidal volume. On the basis of the apparent qualitative differences between Mitragynine and the narcotic analgesic agents in these preliminary studies, it can be anticipated that new chemicals whose structural configurations are quite unlike the morphine-type structure may well produce analgesic properties which are unaccompanied by the limiting side effects of morphine-like drugs.

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References

1. Field, E. *Transactions*
2. Joshi, B. S., Raymoni
3. Zackarias, D. E., Ros
4. Manske, R. H. F. *Th* Press), New York, 196
5. Grewel, K. S. *J. Pha*
6. Grewel, K. S. *Brit. J.*
7. Tjuan, L. C. *Proc. A*
8. Litchfield, J. T. and
9. D'Amour, F. E. and S
10. Hardy, J. D., Wolff Williams and Wilkins
11. De Sanctis, N., Mac (1965).
12. Rinney, D. J. *Statistic* York, (1964).
13. Randall, L. D. and S
14. Eddy, N. B., Leimbac
15. Tedeschi, R. E., Tede Fellows, E. J. *J. Pha*
16. Magnus, R. *Arch. Ges*
17. Deneau, G. A. and Seev 1, 1-8.
18. Cook, L., Weidley, E *Ther.* 113, 11 (1955).
19. Hoffman, W. S. *J. bio*
20. Deneau, G. A. Persons

Received September 30, 1971

References

1. Field, E. *Transactions of the Chemical Society*, 119, 887 (1921).
2. Joshi, B. S., Raymond-Hamet and Taylor, W. I. *Chem. and Ind.*, 573 (1963).
3. Zackarias, D. E., Rosenstein, R. D. and Jeffry, G. A. *Acta Cryst*, 18, 1039 (1965).
4. Manske, R. H. F. *The Alkaloids, Chemistry and Physiology*, 7, 160-163, (Academic Press), New York, 1960.
5. Grewel, K. S. *J. Pharmacol. exp. Ther.* 46, 273 (1932).
6. Grewel, K. S. *Brit. J. Med. Psychol.* 12, 41 (1932).
7. Thuan, L. C. *Proc. Alumni Assoc.*, Malaya, 10, No. 4, Dec. (1957).
8. Litchfield, J. T. and Wilcoxon, F. *J. Pharmacol. exp. Pharmacol.* 96, 99 (1949).
9. D'Amour, F. E. and Smith, D. L. *J. Pharmacol. exp. Ther.* 72, 74 (1941).
10. Hardy, J. D., Wolff, H. G. and Goodell, H. *Pain Sensations and Reactions*, Williams and Wilkins Co., Baltimore, 67-69 (1952).
11. De Sanctis, N., Macko, E., Porter, M., Tedeschi, R. E. *Pharmacologist* 7, 164 (1965).
12. Finney, D. J. *Statistical Methods in Biological Assay*, (Hafner Publishing Co), New York, (1964).
13. Randall, L. D. and Selitto, J. J. *Arch. int. Pharmacodyn.* 111, 409 (1957).
14. Eddy, N. B., Leimbach, D. *J. Pharmacol. exp. Ther.* 107, 385 (1953).
15. Tedeschi, R. E., Tedeschi, D. H., Hitchens, J. T., Cook, L., Mattis, P. A. and Fellows, E. J. *J. Pharmacol. exp. Ther.* 126, 338 (1959).
16. Magnus, R. *Arch. Ges. Physiol.* 102, 123 (1904).
17. Deneau, G. A. and Seevers, M. H. *Bulletin, Drug Addiction and Narcotics*, Addendum 1, 1-8.
18. Cook, L., Weidley, E. F., Morris, R. W. and Mattis, P. A. *J. Pharmacol. exp. Ther.* 113, 11 (1955).
19. Hoffman, W. S. *J. biol. Chem.* 120, 51 (1937).
20. Deneau, G. A. Personal Communication.

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