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Analgetic Activity of a Quinazoline. (32195)

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Recently, a series of quinazoline compounds was found to have analgetic activity that may be characterized as non-narcotic and unique. One of these, 4-(2-dimethylamino-ethylamino)-quinazoline (Fig. 1), designated Su-13026, is the most interesting and its pharmacology will be described here.

Materials and methods. Drugs were prepared as aqueous solutions just prior to testing. Doses given were based on the salts. The following compounds were used: morphine sulfate, aminopyrine, d-amphetamine sulfate (Dexedrine),* d-propoxyphene hydrochloride (Darvon)† and nalorphine hydrochloride (Nalline).‡

Male albino mice (CF-1) weighing 18 to 22 g were used. The ED_{50} values were based on at least 3 logarithmically spaced (0.3 interval) doses per drug. The doses utilized were those for which the range of responses in preliminary tests fell between 16 and 84% (probits 4.0 and 6.0). The best-fitting straight line was determined on logarithmic-probability paper by the method of Litchfield and Wilcoxon(1). All drugs were given orally or subcutaneously at a concentration adjusted for an injection of 0.20 ml per animal.

A constant intensity heat stimulus was used to induce a tail-flick response(2). The apparatus previously described(3) was modified in that a light source with a built-in parabolic reflector (Sylvania, T-12, 150 watts) was focused from a point 5 cm below the tail of the mouse.§ The control reaction time was measured twice in each of 10 animals. To standardize the procedure, the intensity of the stimulus was adjusted so that the control values were between 3.5 and 4.5 seconds. Thus, in a series of 16 experiments the mean ($\pm$ S. D.) control value was 3.95 $\pm$ 0.33 seconds. At each post-drug interval those animals that had a reaction time greater than 1.32 plus the average control value for the group were considered to be "reactors" ($P = <0.001$). The number of reactors at the time of peak drug effect was converted to a per cent value and treated as described above.

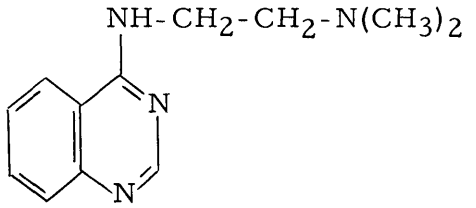
For the interaction studies, paired experiments were done using the tail-flick procedure. The test compound and nalorphine 5 mg/kg or amphetamine 5 mg/kg were given to the first group of 10 mice. The second group re-

* Smith, Kline and French Laboratories, Philadelphia, Pa.

† Lilly Laboratories, Indianapolis, Ind.

‡ Merck, Sharp and Dohme, West Point, Pa.

§ This unit is commercially available from W. A. Schaerr, Brooklyn, N. Y.



Su-13026

4-(2-dimethylaminoethylamino)-quinazoline

FIG. 1.

ceived the test drug and an equal volume of saline. Comparison of the data was made by the X^2-distribution(4).

For the writhing test, mice were divided into 2 groups of 6 mice each. Fifteen minutes before the intraperitoneal injection of the writhing agent, one group was pretreated, subcutaneously or orally, with the test drug while the other group received 0.9% physiological saline by the same route as the test drug. The animals were injected with 300 mg/kg of acetic acid (3% solution) as described by Koster *et al*(5) with modifications by Witkin *et al*(6) and placed in individual containers. This dose caused writhing in all control animals. The total number of writhes per group for the twenty minute period following the acetic acid injection was determined. Based on 322 experiments the mean (± S. D.) control value was 309 ± 23. Each experiment was repeated once so that each point on the dose-response curve represented the results of twelve experimental mice compared with an equal number of controls.

The hot-plate method was as described by Eddy and Leimbach(7). Ten male Wistar rats weighing 150 to 200 g were used in each experiment. Response times were determined once before and at 15-minutes intervals for one hour after drug injection. Only those animals whose control reaction time was less than 13 seconds were used. The mean (± S. D.) control value for a group of 1080 rats was 8.8 ± 2.3 seconds. As in the tail-flick test, the data were quantalized with "reactors" having post-drug reaction time of 16 seconds or greater ($P = <0.01$).

Male albino rabbits weighing 2.2 to 3.0 kg were used in the tooth-pulp studies. The method was essentially the same as that described by Fleisch and Dolivo(8), Yim *et al* (9) and Laffargue(10). Twenty-four to 48 hours prior to the experiment the animals were anesthetized with sodium pentobarbital (25 mg/kg intravenously) and their upper incisor teeth drilled. Stimuli were delivered through a 2-lead stainless steel clip electrode insulated except for 1.0 mm at the tip. A Grass Model S8 stimulator with isolation unit (SIU-4) was used to stimulate the animal with monophasic square waves (1 msec duration, 30 pulses per sec, 1 sec bursts). The minimum voltage necessary to elicit a response 2 out of 3 times was considered the threshold. There was a delay of 20 seconds between each stimulation. Animals that did not respond at 6.0 volts or less were discarded. After 2 threshold determinations at 30 and 15 minutes before drug, the test agent was injected subcutaneously. Post-injection threshold responses were determined at 15, 30, 45, 60, 90 and 120 minutes. The drug effect was expressed as a per cent increase above the average control response at the time of peak effect of the drug.

Respiration, blood pressure, flexor and patellar reflexes were recorded in 15 male cats (2.5 to 3.5 kg) under chloralose-urethane anesthesia. The technique used for monitoring spinal reflexes was as previously described (11) with several modifications. The flexor reflex was recorded by connecting the tendon of the tibialis anticus muscle directly to a Grass Model FT-03 force transducer. The patellar reflex was recorded by connecting a spring from the Achilles tendon to a Statham Model G-1 force transducer. All drugs were administered intravenously at a rate of 1.0 mg/kg/minute. The doses used for d-propoxyphene, Su-13026 and aminopyrine were based on their subcutaneous potency in relation to that of morphine in the tail-flick test. The drug effect on each parameter was the per cent change at time of peak effect.

The 4-hour LD_{50} values were determined for isolated and aggregated mice (5 per con-

TABLE I. Effect of Su-13026 and Other Analgetics in Several Test Procedures.

Compound	Mouse tail-flick* S.C.	Mouse tail-flick* P.O.	Mouse writhing* S.C.	Mouse writhing* P.O.	Rat hot-plate* S.C.	Rabbit tooth-pulp† S.C.
Morphine	7 ± .5	30 ± 7	1.4 ± .2	6 ± 1.4	2 ± .3	2
d-Propoxyphene	16 ± 9	64 ± 20	9 ± 1	31 ± 3	13 ± 2	10
Su-13026	56 ± 7	86 ± 7	24 ± 4	50 ± 9	50 ± 8	30
Aminopyrine	116 ± 9	192 ± 20	52 ±10	105 ±26	200 ±80	75

* ED_{50} ± standard error values (mg/kg).
† Dose (mg/kg) for an approximate 50% elevation of threshold.
S.C. = subcutaneous; P.O. = oral.

TABLE II. Effect of Su-13026 and Other Analgetics in the Anesthetized Cat.

Compound (dose, mg/kg I.V.)	n	Flexnor reflex	Patellar reflex	Blood pressure	Respiration
Morphine (1.0)	4	53 ± 11 ↓	<10	12 ± 5 ↓	38 ± 3 ↓
d-Propoxyphene (2.3)	3	22 ± 3 ↓	"	29 ± 4 ↓	19 ± 3 ↓
Su-13026 (8.0)	4	66 ± 9 ↑	"	<10	<10
Aminopyrine (17.0)	"	102 ± 17 ↑	"	"	"

n = number of animals. S.E. = standard error. I.V. = intravenous. ↑ = increase. ↓ = decrease.

tainer) using opaque plastic 1-liter cylinders. Estimates of the 24-hour LD_{50} values were made with mice housed 10 per cage (6 × 35 × 13 cm) and allowed free access to food and water following drug administration.

Results. Both subcutaneously and orally in the mouse, rat and rabbit the potency of Su-13026 was approximately twice that of aminopyrine (Table I) with the exception being in the rat hot-plate test where the ratio of activity was 4:1. Parenterally, d-propoxyphene was approximately three times as active as Su-13026; orally, however, the former was 1 1/2 times as potent.

At the doses used, amphetamine significantly ($p = <0.001$) *reduced* the effect of Su-13026 in the mouse tail-flick test from 80% to 35% while nalorphine failed to alter this response.

In the anesthetized cat experiments Su-13026 produced a marked increase in the flexor reflex (Table II) with little, if any, effect on the other parameters. Qualitatively this pattern resembled that of aminopyrine. On the other hand, d-propoxyphene caused a depression of the flexor reflex and of respiration as well as a lowering of the blood pressure. This same type of response was produced by morphine.

In the anesthetized dog, Su-13026 at a dose of 9.0 mg/kg intravenously produced approximately a 20% fall in blood pressure that lasted less than 10 minutes. There was no effect on the heart rate or respiration of the animal.

The 4-hour oral LD_{50} value (± S. E.) for Su-13026 was 298 ± 14 mg/kg in isolated mice and only slightly less in aggregated animals, 277 ± 16 mg/kg.

Calculation of oral therapeutic ratios was made utilizing the mouse tail-flick ED_{50} values and the 24-hour LD_{50} estimates. The ratios for Su-13026, d-propoxyphene and aminopyrine were approximtely equal, each being in the range of 4.5 to 5.0.

Discussion. Structure-activity relationship studies in the field of analgetics have led to the development of several interesting theories. Beckett(12), for example, theorized the analgetic receptor as having an anionic site to which the tertiary nitrogen attaches, a flat surface for the planar benzene ring, and a cavity for the bulky portion of the molecule. Braenden *et al*(13) described the central asymmetric carbon atom removed from the nitrogen by a 2-carbon chain for maximum narcotic analgetic activity. The phenolic hydroxyl was also implicated. Unfortunately, such theories are not predictive, since a number of compounds, not analgetics, fulfill these

criteria. They are, however, descriptive since all *narcotic* analgetics satisfy these criteria. In laboratory animals nalorphine antagonizes (14) and amphetamine potentiates(15) the analgetic activity of these compounds. Su-13026 does not have an asymmetric carbon atom, it has no anionic group; in short, it does not fulfill the sterochemical requirements for a narcotic analgetic. In laboratory animals, its analgetic effect *is not* antagonized by nalorphine or potentiated by amphetamine. Su-13026 does not reduce blood pressure or respiratory rate. Furthermore, it is not addicting in monkeys (Seevers, personal communications). Hence, this compound may be classified as a non-narcotic analgetic.

The pharmacological profile of Su-13026 indicates that this compound is a unique analgetic. Anti-inflammatory activity, a characteristic property of aminopyrine and the salicylates, was not produced by Su-13026. Muscle relaxants, such as carisoprodol(16) and phenyramidol(17) have been reported to have analgetic activity. The absence of a paralyzing action and a stimulant, rather than a depressant, effect on spinal reflexes would suggest that Su-13026 is not a muscle-relaxant type of analgetic. Frommel *et al*(18) reported that chlorpromazine had analgetic activity in guinea pigs; the failure of Su-13026 to produce a "tranquilizing" effect would preclude its classification as a phenothiazine-type analgetic. Sympathomimetic amines, particularly amphetamine, have been reported to have analgetic activity in humans(19). Randall *et al* (20) demonstrated a pain threshold elevation with amphetamine in rats but noted strong central excitation at the effective analgetic level. Considering the stimulant effect of Su-13026 in intact animals and in anesthetized cats, and its positive effect in the tail-flick test, it would appear that Su-13026 might be classified as an amphetamine-like analgetic. However, the toxicity of Su-13026 in mice was not increased by aggregation, nor was the effect of morphine in the tail-flick test potentiated by Su-13026.

The antagonism of the effect of Su-13026 by amphetamine in the tail-flick test may be explained on the basis of the excitant properties of these two compounds interacting such that the animals were hypersensitive to the stimulus.

Summary. Four test systems were used to demonstrate the analgetic activity of Su-13026, a quinazoline compound, in mice, rats and rabbits. Signs of central nervous system stimulation were noted. Respiration was not depressed in any of the species studied. Nalorphine did not antagonize, nor did amphetamine potentiate, the activity of this compound in mice. Results indicate that this compound has a safe therapeutic ratio and is non-narcotic.

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Serologic Response in Man to Adenovirus and SV_{40} Components In Adenovirus Vaccines.* (32196)

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This report summarizes the findings in a retrospective study concerned with the serological response in man following vaccination with adenovirus vaccines prepared with adenovirus strains containing SV_{40} genetic material. The SV_{40} component was enclosed within adenovirus capsids; no detectable SV_{40} infectious virus was present in the vaccines or the seed strains. An earlier report from one of our laboratories presented findings, based on studies in hamsters concerning the occurrence of SV_{40} neoplastic and antigenic information in adenovirus type 3, strain JF, one of the virus strains employed in the manufacture of vaccine used in the current study(1).

It was reported earlier that when the vaccines employed in this investigation were given to Naval recruits, there was an approximate 50% reduction in hospitalized acute respiratory disease admission rates as compared with the rates of adenovirus disease in recruits in an unvaccinated control group (2).

Materials and methods. Vaccines used were live oral monovalent adenovirus type 4, an inactivated parenteral monovalent adenovirus type 4 and an inactivated preparation of parenteral trivalent adenovirus types 3, 4, and 7. The live type 4 vaccine was developed and described by Dr. R. M. Chanock (3). It was commercially manufactured from virus seed that had been passed only in human cell cultures. The seed material was free of SV_{40} virion and SV_{40} genetic information and devoid of oncogenicity when examined in appropriate tests(3). The vaccine was lyophilized, placed in enteric coated capsules and kept at —20 for 6 months until used.

The inactivated type 4 vaccine was prepared commercially in the conventional manner(4) in African green monkey kidney cells from a virus strain recovered in 1962 by Dr. E. L. Buescher, and subsequently passed 15 times in African green monkey kidney cells. At this passage level the virus was shown to be free of infectious SV_{40}, both in the laboratory of the manufacturer and in our laboratory. Additionally, it was demonstrated that the virus did not induce SV_{40} "T" antigen in cell cultures or in hamsters and accordingly it was assumed to be free of SV_{40} genetic material. These tests were performed with an aliquot of the virus pool which was removed for examination before the pool was inactivated for use in the manufacture of the monovalent type 4 vaccine. A portion of the same inactivated monovalent pool was used in the manufacture of the type 4 component of the trivalent types 3, 4, and 7 vaccine. The histories of the other 2 adenovirus components in the trivalent vaccine, *i.e.*, type 3, strain JF and type 7, strain LL are recorded(1,5) and are known to have been propagated early in their passage history in cultures of rhesus monkey kidney cells which were contaminated with SV_{40}. Subsequently, both strains were passed in the presence of SV_{40} antiserum and this procedure

* The field aspects of this study were part of a larger investigation of adenovirus vaccines performed by personnel of NAMRU #4, R. O. Peckinpaugh, Officer in Charge; M. Rosenbaum, E. E. Edwards and W. E. Pierce, Chiefs of Virology, Immunology and Biometric Divisions, respectively.