J. Pharm. Pharmacol., 1965, **17**, 509–516 Received January 25, 1965

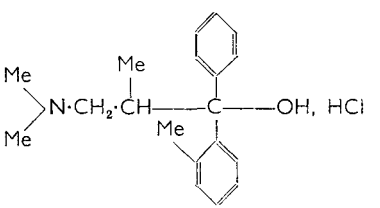
Pharmacological properties of 3-dimethylamino-2-methyl-1-phenyl-1-*o*-tolylpropanol (SK&F 70463-A)

D. I. BARRON,* G. H. HALL,† I. L. NATOFF AND D. K. VALLANCE*

3-Dimethylamino-2-methyl-1-phenyl-1-*o*-tolylpropanol hydrochloride (SK&F 70463-A) produces both stimulant and depressant effects in laboratory animals. In particular, it possesses marked anticonvulsant activity. Diuretic effects have also been observed.

IN examining a series of diarylpropanolamines of general formula $R^1R^2{\cdot}N{\cdot}CH_2{\cdot}CHR^3{\cdot}CAr^1Ar^2OH$ which possess both diuretic and anticonvulsant activity, our interest at first centred on the diuretic properties of the compounds and various structural modifications were made to enhance this activity. However, it was found that the diuretic effects were always accompanied by effects on the central nervous system and these came to be regarded as the most interesting feature.

3-Dimethylamino-2-methyl-1-phenyl-1-*o*-tolylpropanol hydrochloride



(SK&F 70463-A)

(SK&F 70463-A) proved to be the most active compound in the series and was therefore selected for a more detailed investigation of its pharmacological properties. Its activity in various tests was compared with that of suitable reference compounds, including diphenylhydantoin, imipramine, chlorpromazine and amphetamine.

Methods

General. Male Schofield albino mice, approximately 20 g, male Wistar and Sprague Dawley albino rats, 100–160 g, cats, 2–4 kg, mongrel and beagle dogs, approximately 10 kg, and rhesus monkeys, approximately 2 kg, were used. The compounds, dissolved, or suspended in 5% acacia were administered by stomach tube at varying dose levels to groups of 20 animals, unless otherwise stated. LD50 or ED50 values and their confidence limits ($P = 0{\cdot}95$) were calculated using the method of Litchfield & Wilcoxon (1949). Dose volumes were adjusted to 2·5 ml/100 g for mice and rats, and 2 ml/kg for dogs and monkeys. Cats were given the compounds in hard gelatine capsules.

From the Pharmacology Department, Smith Kline & French Laboratories Ltd., Welwyn Garden City, Hertfordshire.

Present address: * The Biological Research Department, The British Drug Houses Ltd., Godalming, Surrey. † The Tobacco Research Council Laboratories, Otley Road, Harrogate, Yorkshire.

Acute toxicity. The compounds were administered to mice and rats and the LD50 values calculated from the total mortalities occurring in the seven days after dosing.

Behavioural studies. Behavioural studies were undertaken using cats, mongrel dogs and monkeys. In these species, changes in behaviour and condition of the animals were recorded both on the day of the experiment and, if necessary, on subsequent days. One animal was used at each dose level.

Prevention of reserpine-induced ptosis. The method was based on that used by Costa, Garattini & Valzelli (1960) to demonstrate antagonism of reserpine-induced ptosis by imipramine in rats. Reserpine (1·5 mg/kg) was injected intravenously 2 hr after administration of the compounds to Wistar rats. The ED50 value was calculated from the numbers of rats without ptosis after a period of 45 min, ptosis being defined as not less than $\frac{2}{3}$ closure of the palpebral fissure persisting for more than 15 sec.

Potentiation of picrotoxin convulsions. The procedure was based on an observation by Tedeschi (personal communication) that central nervous system stimulants (e.g., dexamphetamine) and antidepressants (e.g., imipramine, tranylcypromine) potentiate a sub-threshold dose of picrotoxin to cause facial or forelimb clonus or both. Sprague-Dawley rats were injected intravenously with an aqueous solution of picrotoxin (1·35 mg/kg) 2 hr after receiving the compounds. They were then observed for 30 min and the ED50 calculated from the numbers of animals with clonus.

Stimulation of confinement motor activity. Two hr after dosing with the compounds, Wistar rats were placed in Confinement Motor Activity Units (Tedeschi, Fowler, Cromley, Pauls, Eby & Fellows, 1964) which detect minor changes in locomotor activity such as are produced by caffeine and tranylcypromine. Following an acclimatization period of 5 min, two successive 10 min activity counts were recorded. Mean activity counts for each period were calculated and the logarithms of these values plotted against log dose. The Stimulant Dose 200 (SD 200) for each period was determined graphically and is defined as that dose of a compound which increases by 200% the mean 10 min activity count of control animals tested concomitantly.

Anorexic activity. Wistar rats were trained to consume the normal daily requirement of food within 6 hr. During the training period, lasting 14 days, the animals were housed in individual cages. Food (Diet 41B cubes) was supplied in excess at 10 am each day and the surplus removed at 4 pm. Trained animals were then dosed with the test compounds or the vehicle alone and 1 hr later were presented with a weighed amount of food (approximately 5 g) for a period of 1 hr. Any food remaining was then removed, weighed and the food consumption calculated. The mean food consumption for each treated group was expressed as a percentage of that for the control group and plotted against log dose. The dose required to reduce the food consumption to 50% of the control value was then determined.

Protection against amphetamine toxicity in aggregated mice. The method was based on the studies of Lasagna & McCann (1957). Ten mice were used for each dose group and 1 hr after administration of the compounds the mice were given (±)-amphetamine sulphate (20 mg/kg) by subcutaneous injection. The animals were then placed two groups to a cage in a constant temperature cabinet maintained at approximately 27°. The number surviving in each dose group was recorded 24 hr later and the ED50 calculated.

TABLE 1. COMPARISON OF SK&F 70463-A AND VARIOUS REFERENCE DRUGS IN TESTS FOR CENTRAL NERVOUS SYSTEM ACTIVITY AFTER ORAL ADMINISTRATION

Test	Parameter	SK&F 70463-A mg/kg	Dexamphetamine sulphate mg/kg	Imipramine hydrochloride mg/kg	Chlorpromazine hydrochloride mg/kg	Diphenylhydantoin mg/kg
Acute toxicity (mice)	LD50	245 (211–284)	110 (85–143)	324 (289–363)		
Acute toxicity (rats)	LD50	185 (145–237)	210 (159–277)	490 (398–603)		
Prevention of reserpine-induced ptosis	ED50	27 (17–43)		3·6 (2·5–5·3)		
Potentiation of picrotoxin convulsions	ED50	16 (10–24)	10 (6·6–15)	34 (23–51)		
Anorexic activity	Dose giving 50% reduction in food consumption	13	2·1			
Confinement motor activity: 1st count: 2nd count:	SD200 SD200	19 13	1·8 1·9			
Protection against amphetamine toxicity (aggregated mice)	ED50	18 (14–23)		54 (35–83)	2·4 (2·0–2·8)	
Block of conditioned avoidance response	ED50	11 (8·8–14)			6·0 (4·7–7·7)	
Block of unconditioned avoidance response	ED50	16 (13–20)			23 (14–36)	
Prevention of apomorphine-induced chewing	ED50	7·5 (4·2–13)			4·7 (3·4–6·6)	
Potentiation of hexobarbitone sleeping time	Dose giving 600% increase	31·6			5·8	
Prevention of maximal electroshock seizures	ED50	2·4 (1·7–3·3)		30 (22–41)		2·4 (1·9–3·1)
Prevention of maximal leptazol seizures	ED50	3·6 (2·7–4·9)		95 (65–124)		6·6 (4·7–9·2)

Confidence limits (P = 0·95) are shown in parentheses

Blockade of a conditioned avoidance response. The method was essentially that described by Cook & Weidley (1957) using Wistar rats. At 1, 2 and 3 hr after dosing with the compounds, previously conditioned animals were subjected to the sound of the buzzer. Failure to climb the pole within 30 sec was indicative of blockade of the conditioned response. They were then subjected to the shock and buzzer together for 30 sec and failure to climb the pole under these conditions indicated that the unconditioned response was blocked. The ED50 values for blockage of both the conditioned and the unconditioned responses were calculated.

Potentiation of hexobarbitone sleeping time. Two hr after dosing with the test compounds, mice were injected intravenously with a solution of hexobarbitone sodium (40 mg/kg) in normal saline. Each animal was immediately placed on its back or side and left until it succeeded in righting itself, the sleeping time being noted. The mean sleeping time for each treatment group was expressed as a percentage increase over that of controls given the vehicle alone. These values were plotted against log dose and the dose causing a 600% increase in sleeping time was determined.

Prevention of apomorphine-induced chewing. The procedure followed was essentially that described by Janssen, Niemegeers & Jageneau (1960). Two hr after dosing with the compounds, Wistar rats were injected intravenously with apomorphine (0·25 mg/kg) and were then observed for a further 30 min. The ED50 was calculated from the numbers in which licking or chewing or both did not occur.

Prevention of apomorphine-induced vomiting. Female beagles were injected once weekly for three consecutive weeks with apomorphine hydrochloride (40 μg/kg, intravenously). This treatment produced emesis in all dogs at each trial within 2–3 min. The animals were dosed with the test compound during the fourth week, two animals being used at each dose level. The animals were fed 2 hr after dosing and apomorphine was injected 1 hr later. The production or prevention of emesis was noted.

Maximal electroshock seizure test. The prevention of maximal electroshock seizures in mice was determined by a modification of the method of Swinyard, Brown & Goodman (1952), the current strength being reduced to 25 mA. ED50 values were estimated from the numbers of mice in which tonic extension of the hind limbs was prevented at 1, 2 or 3 hr after dosing.

Maximal leptazol seizure test. The procedure was essentially as described by Goodman, Grewal, Brown & Swinyard (1953), the test compounds being administered to mice 2 hr before the intravenous injection of leptazol (60 mg/kg). ED50 values were estimated from the numbers of animals in which tonic extension of the hind limbs was prevented.

Diuretic activity. Wistar rats, 110–220 g, were uniformly distributed by weight into groups of eight. The compounds were administered as solutions, or suspensions in 5% (w/v) acacia in normal saline, control animals receiving the vehicle alone. Each group was then placed in a metabolism cage standing over a stainless steel funnel and the urine was collected for 5 hr after which urine volume and pH were recorded, and aliquots of each sample were taken for electrolyte analysis. The concentrations of sodium and potassium ions were estimated by flame photometry and of chloride ions by the iodimetric method of Sendroy (1937). The residual anion excretion was calculated by subtraction of the chloride ion excretion from the total cation excretion.

Inhibition of carbonic anhydrase. The procedure *in vitro* was that described by Maren (1960), canine erythrocytes being used as the enzyme source. *In vivo* assessment was made with mice, 20–30 g, divided into

groups of ten. One hr after dosing with the test compounds, the mice were killed by dislocating the neck, and the brains and kidneys were removed, rinsed in chilled normal saline, dried between blotting paper, and weighed. The tissues were homogenised in nine volumes of 0·25M sucrose, and the homogenates cleared by precipitation of proteins with 50% saturated ammonium sulphate. The carbonic anhydrase activity of the cleared homogenates was then determined as above, the volume of homogenate containing one unit of enzyme activity (i.e., the volume required to induce a colour change in the phenol red indicator in half the time taken for a boiled aliquot of the homogenate to produce a similar colour change) being obtained. The number of units per gram of tissue was calculated.

Results

Acute toxicity. The acute toxicities of SK&F 70463-A, imipramine hydrochloride and dexamphetamine sulphate in the mouse and rat are shown in Table 1.

Behavioural studies. After the administration of SK&F 70463-A to cats, dogs and monkeys, the first effects were stimulation of the central nervous system, typified by tremors and convulsions. These were followed by prostration at higher doses, and several of the animals remained in a severely depressed state for several days. Complete recovery was observed after a dose of 25 mg/kg in cats, 10 mg/kg in dogs, and 40 mg/kg in monkeys.

Prevention of reserpine-induced ptosis. SK&F 70463-A has approximately one-seventh of the activity of imipramine hydrochloride (see Table 1).

Potentiation of picrotoxin convulsions. The activity of SK&F 70463-A in this test, as shown in Table 1, is approximately twice that of imipramine hydrochloride and two-thirds that of dexamphetamine sulphate.

Stimulation of confinement motor activity. SK&F 70463-A produces stimulation of confinement motor activity but is less active than dexamphetamine sulphate (see Table 1). It appeared to have a greater effect on activity during the second period of observation than during the first. Tedeschi & others (1964) have reported a similar finding with amphetamine but this was not confirmed by us.

Anorexic activity. SK&F 70463-A has approximately one-sixth of the activity of dexamphetamine sulphate (Table 1). It appears that the ratios of the doses effective in the anorexic and confinement motor activity tests are approximately the same for the two compounds, suggesting possibly a similar mechanism of action. This is supported by parallelism of the dose-response curves.

Protection against amphetamine toxicity in aggregated mice. The activity of SK&F 70463-A in this test has been compared with that of the major tranquilliser, chlorpromazine hydrochloride, and the antidepressant, imipramine hydrochloride. The results in Table 1 indicate that it possesses an intermediate degree of activity, being one-eighth as active as chlorpromazine but three times as active as imipramine.

Blockade of a conditioned avoidance response. Although SK&F 70463-A is capable of blocking a conditioned avoidance response and is approximately one-half as active as chlorpromazine hydrochloride (see Table 1), this appears to be a relatively non-specific effect. Not only was the conditioned response blocked but so also was the unconditioned response at a dose level only marginally greater. This is in contrast to the results with chlorpromazine, with which there was an approximately fourfold difference in doses in this case. It was evident that the animals given SK&F 70463-A were markedly excited and inability to climb the pole would seem to have been a measure of a state of agitation rather than of tranquillisation.

TABLE 2. EFFECT OF SK&F 70463-A, CHLOROTHIAZIDE AND ACETAZOLAMIDE ON WATER AND ELECTROLYTE EXCRETION FOLLOWING ORAL ADMINISTRATION TO SALINE-LOADED RATS

Compound	Dose mg/kg	No. of rats	Mean body weight (g) and range	Urinary pH	Water excretion (ml/kg/5 hr)	Electrolyte excretion (m-equiv./kg/5 hr)				
						Na	K	Cl	HCO_3	Na/K
—	—	24	162·8 (115–211)	6·05 5·75 5·70	8·42	1·77	0·81	2·25	0·33	2·20
Chlorothiazide	5	24	163·4 (122–218)	6·00 5·85 5·70	15·30	3·05	1·03	3·81	0·27	2·95
	15	24	165·0 (125–217)	6·10 5·65 5·80	20·70	4·10	1·25	5·41	0·07	3·29
Acetazolamide	1	24	162·8 (115–209)	7·00 6·90 6·70	12·78	2·46	1·05	2·56	0·95	2·35
	3	24	164·1 (119–214)	7·65 7·50 7·45	16·37	3·43	1·28	3·40	1·31	2·69
SK&F 70463-A	10	24	164·5 (114–210)	6·50 6·00 6·25	12·16	2·46	1·08	2·95	0·59	2·28
	20	24	163·4 (119–215)	6·90 6·40 7·20	19·63	2·89	0·98	3·09	0·78	2·93

Potentiation of hexobarbitone sleeping time. SK&F 70463-A is approximately one-fifth as active as chlorpromazine hydrochloride (see Table 1).

Prevention of apomorphine-induced chewing. The activity of SK&F 70463-A is approximately two-thirds that of chlorpromazine hydrochloride (see Table 1).

Prevention of apomorphine-induced vomiting. No activity against apomorphine-induced emesis in dogs could be demonstrated with SK&F 70463-A at doses up to 10 mg/kg. Higher doses were not examined because of the likelihood of clonic convulsions and prostration. This result is of particular interest since Janssen & others (1960) reported that all compounds which they had found to be active in preventing apomorphine-induced chewing in the rat also prevented the emetic effect of apomorphine in the dog.

Maximal electroshock seizure test. SK&F 70463-A was found to be equal in activity to diphenylhydantoin and twelve times as active as imipramine hydrochloride (see Table 1).

Maximal leptazol seizure test. SK&F 70463-A is slightly less effective against leptazol than against electroshock seizures (see Table 1), but it is almost twice as active as diphenylhydantoin and more than twenty-five times as active as imipramine hydrochloride.

Diuretic activity. The results of three experiments comparing SK&F 70463-A with chlorothiazide and acetazolamide have been combined and are summarised in Table 2. The slope of the log dose-urinary volume response curve for SK&F 70463-A differs from those of the reference compounds, so that an estimation of the relative potencies is not possible. Parallel log dose-response curves are, however, obtained with sodium output and it appears that SK&F 70463-A has one-thirteenth of the natriuretic activity of acetazolamide and one-fifth that of chlorothiazide. Qualitatively, whereas chlorothiazide increased the urinary excretion of water, sodium, potassium and chloride, SK&F 70463-A and acetazolamide also increased excretion of "residual anion" (presumably bicarbonate). SK&F 70463-A had the least effect on potassium excretion and the loss was less at the higher dose than at the lower.

TABLE 3. EFFECT OF SK&F 70463-A AND ACETAZOLAMIDE ON CARBONIC ANHYDRASE ACTIVITY *IN VITRO*

	Concentration (μM) of added compound	Time (sec) for colour change
Enzyme preparation alone	—	10
Enzyme preparation	6·5	39
plus acetazolamide	650	40
Enzyme preparation	4·0	11
plus SK&F 70463-A	400	16
Boiled enzyme preparation alone ..	—	40

Inhibition of carbonic anhydrase: in vitro. Table 3 records the time of passage of a constant flow of carbon dioxide gas until the indicator changed colour. Whilst acetazolamide completely abolished enzyme activity at a concentration of 6·5 μM, the time being increased to that observed when the enzyme preparation was inactivated by boiling, SK&F 70463-A was without effect at concentrations up to 400 μM.

TABLE 4. EFFECT OF SK&F 70463-A AND ACETAZOLAMIDE ON THE CARBONIC ANHYDRASE ACTIVITY OF MOUSE KIDNEY AND BRAIN

Drug	Dose (mg/kg) orally	No. of units of enzyme activity per g tissue	
		Kidney	Brain
—	—	420	480
Acetazolamide ..	30	40	80
SK&F 70463-A ..	30	350	360

In vivo. The carbonic anhydrase activity of tissues obtained from mice treated with acetazolamide or SKF 70463-A is presented in Table 4. Acetazolamide (30 mg/kg) caused a marked reduction in activity whereas a similar dose of SK&F 70463-A had relatively little inhibitory effect on the carbonic anhydrase of either kidney or brain.

Discussion

It is apparent that SK&F 70463-A produces a mixture of stimulant and depressant effects on the central nervous system. The results of the anti-reserpine, picrotoxin potentiation, confinement motor activity and anorexic tests, together with the gross observations made in the behavioural studies, indicate that the compound has CNS stimulant effects which appear to resemble those of amphetamine. However, the duration of hexobarbitone anaesthesia in mice was prolonged. A number of other tests for CNS depressant effects (anti-amphetamine, conditioned avoidance response, anti-apomorphine, maximal electroshock seizure, maximal leptazol seizure) also gave positive results, and it becomes evident, therefore, that the compound has a spectrum of pharmacological activity which, resembles to some extent that of imipramine hydrochloride.

In the diuretic studies, the increased pH of the urine, together with the increase in "residual anion" excretion suggested that SK&F 70463-A might act by inhibition of carbonic anhydrase. The carbonic anhydrase inhibitor acetazolamide has been used successfully in the treatment of epilepsy (Goodman & Gilman, 1954) and there is evidence (Millichap & Woodbury, 1954) that its anticonvulsant activity is a direct result of the inhibition of brain carbonic anhydrase. Since SK&F 70463-A also possesses marked anticonvulsant activity, the possibility existed that both its diuretic and anticonvulsant properties could likewise be due to carbonic anhydrase inhibition. Carbonic anhydrase studies *in vitro* and *in vivo* fail to support this hypothesis.

The main clinical effects reported were ataxia and drowsiness and when stimulant activity was observed it closely resembled that due to amphetamine but the incidence of side-effects was so high that investigations of the potential anticonvulsant actions of SK&F 70463-A were not made.

References

Cook, L. & Weidley, E. (1957). *Ann. N.Y. Acad. Sci.*, **66**, 740–752.
Costa, E., Garattini, S. & Valzelli, L. (1960). *Experientia*, **16**, 461–463.
Goodman, L. S. & Gilman, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed. p. 856, New York: Macmillan.
Goodman, L. S., Grewal, M. S., Brown, W. C. & Swinyard, E. A. (1953). *J. Pharmacol.*, **108**, 168–176.
Janssen, P. A. J., Niemegeers, C. J. C. & Jageneau, A. H. M. (1960). *Arzneimitt.-Forsch.*, **10**, 1003–1005.
Lasagna, L. & McCann, W. P. (1957). *Science*, **125**, 1241–1242.
Litchfield, J. T. & Wilcoxon, F. (1949). *J. Pharmacol.*, **96**, 99–113.
Maren, H. (1960). *Ibid.*, **130**, 26–29.
Millichap, J. G. & Woodbury, D. M. (1954). *Presented at Amer. Soc. Pharmacol.*, Charlottesville, Va., September 6–8.
Sendroy, Jr., J. (1937). *J. biol. Chem.*, **120**, 405–417.
Swinyard, E. A., Brown, W. C. & Goodman, L. S. (1952). *J. Pharmacol.*, **106**, 319–330.
Tedeschi, D. H., Fowler, P. J., Cromley, W. H., Pauls, J. F., Eby, R. Z. & Fellows, E. J. (1964). *J. pharm. Sci.*, **53**, 1046–1050.
Wiebelhaus, V. D., Brennan, F. T., Sonowski, G. F. & Polk, A. K. (1960). *Fed. Proc.*, **19**, 364.