

R48

GENERAL PHARMACOLOGY

Octopirox = H 72 6146 A

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## INTRODUCTION

Octopirox which is to be used as an active ingredient in shampoos and scalp lotions was submitted to pharmacological screening. A dose of 5 mg/kg was selected for all routes. This dose corresponds to the 10- to 15-fold amount per kg body weight available for absorption in man. Washing tests revealed that maximally 20 mg substance remain on the scalp and hair. The pharmacological studies were designed to preclude as far as possible conceivable acute effects on physiological functions following absorption or unintended consumption of the substance.

Examinations of the effect of Octopirox on the central nervous system, vegetative functions (isolated organs), cardiovascular parameters, and specific metabolic functions gave no evidence that the compound has acute pharmacological effects in animal experiments.

Signed:

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1. EFFECT ON CENTRAL NERVOUS SYSTEM

1.1 Effect of oral Octopirox on spontaneous behavior in mice

Method

Groups of 6 male NMRI mice (Ivanovas) received orally 5 - 1,000 mg Octopirox per kg body weight by stomach tube. The compound was dissolved in methylcellulose suspension in a volume of 10 ml/kg. The control animals were given only methylcellulose suspension. During the first few hours after administration, the mice were observed continuously after a screening scheme of S. Irwin.

Results

<u>Octopirox mg/kg</u>	<u>Symptoms</u>
5	after all doses, unremarkable
300	behaviour, no difference vs.
1,000	control. No death.

Conclusion

Oral Octopirox in doses between 5 and 1,000 mg/kg did not cause behavioral changes in mice and was not toxic.

1.2 Effect of oral Octopirox on spontaneous motility increased by methamphetamine in mice

Method

The principle of intermittent observation after L. Ther, Dtsch. Apoth. Ztg. 17, 292 (1953) was employed. Two groups of 3 male mice (Ivanovas, 22 - 25 g) were orally pretreated with 5 mg Octopirox per kg body weight by stomach tube 30 minutes before the beginning of activity counting. The compound was dissolved in methylcellulose suspension (10 ml/kg). Two control groups were only treated with methylcellulose suspension. Twenty minutes later, the animals received a subcutaneous injection of 0.5 mg methamphetamine per kg body weight. Under these test conditions, a maximum of 60 counts per mouse was achieved which indicated strong motor activity. Values around 0 indicated a suppression of the central nervous system.

Evaluation: Rank sum test (U-test) after Wilcoxon, Mann, and Whitney.

Results

Rank sum R (of the smaller group)		Critical region U
Control	$n_1 = 6$	
Octopirox	$n_2 = 6$	
5 mg/kg	$R_2 = 9$	
	n. s.	$U(6,6,0.05) = 5$

Conclusion

Oral Octopirox administered in a dose of 5 mg/kg to mice did not suppress the motor activity increased by methamphetamine.

1.3 Effect of oral Octopirox on hexobarbital-induced sleeping time in mice

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Method

Groups of 10 male NMRI mice (Ivanovas, 23 - 26 g) per dose were used. The test group received orally 5 mg Octopirox per kg body weight by stomach tube, dissolved in methylcellulose suspension in a volume of 10 ml/kg. The control group was given only methylcellulose suspension. One hour later, the mice were given an i.v. injection of 55 mg hexobarbital soluble. The duration of time spent in lateral position (= sleeping time) was assessed. The prolongation of sleeping time is given in percent of control.

Results

Treatment	S l e e p i n g t i m e	
	$\bar{x} \pm s$ (min)	% of control
Control	20 $\pm$ 19.47	100
Octopirox 5 mg/kg	18 $\pm$ 18.89	90

Conclusion

Oral Octopirox administered in a dose of 5 mg/kg did not prolong hexobarbital-induced sleeping time in mice.

1.4 Effect of oral Octopirox on experimentally induced convulsions in mice

1.4.1 Effect on pentylenetetrazole-induced convulsions in mice

Method

Groups of 10 male NMRI mice (Ivanovas, 21 - 24 g) per dose were used. The test group was orally pretreated with 5 mg Octopirox per kg body weight, dissolved in methylcellulose suspension in a volume of 10 ml/kg. The control group was given only methylcellulose suspension. Thirty minutes later, pentylenetetrazole was administered intravenously as a 1 percent aqueous solution at a rate of 0.2 ml/min. Three parameters were observed: time (sec.) until occurrence of persistent convulsions, presence of tonic extensor convulsions, and time (sec.) until occurrence of death.

Results

Treatment	Persistent convulsions $\bar{x} \pm s$ (sec)	Tonic extensor convulsions	Death $\bar{x} \pm s$ (sec)
Control	59 $\pm$ 29.5	10/10	73 $\pm$ 32.0
Octopirox 5 mg/kg	47 $\pm$ 25.3	9/10	65 $\pm$ 27.8

Conclusion

Oral Octopirox administered in a dose of 5 mg/kg did not reveal a protective effect against pentylenetetrazole-induced convulsions in mice.

#### 1.4.2 Effect on electroshock-induced convulsions in mice

##### Method

Male NMRI mice (Gassner, 23 - 27 g) were orally pretreated with 5 mg Octopirox per kg body weight, dissolved in methylcellulose suspension in a volume of 10 ml/kg. The control animals were given only methylcellulose suspension. A positive control was treated with oral diazepam. The electroshock was produced by application of a current of 20 ma and 50 Hz for 0.2 sec by means of corneal electrodes.

##### Results

Treatment	n	Tonic extensor convulsions
Control	10	10/10
Octopirox 5 mg/kg	30	28/30
Diazepam 2 mg/kg	10	5/10

##### Conclusion

Oral Octopirox administered in a dose of 5 mg/kg did not have an anticonvulsive effect in the model of maximal electroshock convulsions in mice.

1.5 Effect of oral Octopirox on tetrabenazine-induced ptosis  
in mice

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Method

Groups of 10 male mice (Ivanovas, 20 - 26 g) per dose were used. The test group received orally 5 mg Octopirox per kg body weight by stomach tube, dissolved in methylcellulose suspension in a volume of 10 ml/kg. The control animals were given only methylcellulose suspension. A positive control was given imipramine 20 mg/kg. One hour later, the mice were treated intraperitoneally with tetrabenazine 30 mg/kg. The antagonism against tetrabenazine-induced ptosis was assessed.

The following point scheme was used for semi-quantitative evaluation of ptosis:

- 2 eyelid completely closed
- 1 eyelid half closed
- 0 eyelid completely open

The animals were observed 30 and 60 minutes after administration of tetrabenazine.

Results

Treatment	Minutes after tetrabenazine treatment	
	30	60
Control	2.0	2.0
Octopirox 5 mg/kg	1.8	2.0
Imipramine 20 mg/kg	0.1	0.3

1.3 Effect of oral Octopirox on hexobarbital-induced sleeping time in mice

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Method

Groups of 10 male NMRI mice (Ivanovas, 23 - 26 g) per dose were used. The test group received orally 5 mg Octopirox per kg body weight by stomach tube, dissolved in methylcellulose suspension in a volume of 10 ml/kg. The control group was given only methylcellulose suspension. One hour later, the mice were given an i.v. injection of 55 mg hexobarbital soluble. The duration of time spent in lateral position (= sleeping time) was assessed. The prolongation of sleeping time is given in percent of control.

Results

Treatment	S l e e p i n g t i m e	
	$\bar{x} \pm s$ (min)	% of control
Control	20 $\pm$ 19.47	100
Octopirox 5 mg/kg	18 $\pm$ 18.89	90

Conclusion

Oral Octopirox administered in a dose of 5 mg/kg did not prolong hexobarbital-induced sleeping time in mice.

1.4 Effect of oral Octopirox on experimentally induced convulsions in mice

1.4.1 Effect on pentylenetetrazole-induced convulsions in mice

Method

Groups of 10 male NMRI mice (Ivanovas, 21 - 24 g) per dose were used. The test group was orally pretreated with 5 mg Octopirox per kg body weight, dissolved in methylcellulose suspension in a volume of 10 ml/kg. The control group was given only methylcellulose suspension. Thirty minutes later, pentylenetetrazole was administered intravenously as a 1 percent aqueous solution at a rate of 0.2 ml/min. Three parameters were observed: time (sec.) until occurrence of persistent convulsions, presence of tonic extensor convulsions, and time (sec.) until occurrence of death.

Results

Treatment	Persistent convulsions $\bar{x} \pm s$ (sec)	Tonic extensor convulsions	Death $\bar{x} \pm s$ (sec)
Control	59 $\pm$ 29.5	10/10	73 $\pm$ 32.0
Octopirox 5 mg/kg	47 $\pm$ 25.3	9/10	65 $\pm$ 27.8

Conclusion

Oral Octopirox administered in a dose of 5 mg/kg did not reveal a protective effect against pentylenetetrazole-induced convulsions in mice.

Conclusion

Oral Octopirox administered in a dose of 5 mg/kg did not influence tetrabenazine-induced ptosis in mice.

1.6 Effect of oral Octopirox on thermal pain in mice  
(tail-flick test)

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Method

After the method of L. Ther and coworkers, Dtsch. Apoth. Ztg. 103, 514 (1963), pain was produced by directing radiant heat onto the tail of male NMRI mice which responded by escape or defense reactions within a few seconds. The study was conducted on two groups each of 10 animals in an appropriate apparatus consisting of a restrainer for the mice and a heat source (halogen lamp with a concave mirror). The latter was connected with an electric stop-watch.

After having measured the normal pain reaction time, the test compound was administered orally in a dose of 5 mg/kg. Thirty and 60 minutes after administration, the reaction time to thermal pain was measured again. A prolongation of the reaction time by 100 percent was taken as evidence of an analgesic effect.

Results

Octopirox	Reaction time to thermal pain (sec) $\bar{x}$		
	Before drug	After drug	
		30 min	60 min
5 mg/kg	4.3	4.2	4.4
5 mg/kg	4.2	4.8	4.3

Conclusion

Oral Octopirox administered in a dose of 5 mg/kg to mice did not produce an analgesic effect in the tail-flick test.

2. EFFECT ON ISOLATED ORGANS

## 2.1 Effect of Octopirox on the isolated guinea pig ileum

### Method

The experiments were performed after the conventional suspension technique by Magnus (Pflüger's Arch. ges. Physiol. 102, 123 (1904)). The ileum sections were mounted in Tyrode's solution (carbogen-saturated) at 37°C and attached to isotonic levers for recording of the contractions.

The test compound was examined for antagonism to the following spasmogens: carbachol, final concentration  $4 \times 10^{-8}$  g/ml bath, histamine x 2 HCl, final concentration  $2 \times 10^{-7}$  g/ml bath, BaCl<sub>2</sub>, final concentration  $10^{-4}$  g/ml bath. Octopirox was added to the organ bath in a final concentration of  $10^{-5}$  g/ml two minutes before addition of the spasmogens (two experiments). Contraction by the spasmogens alone = 100 %.

### Results

Spasmogen	Changes in % of initial value after prior addition of Octopirox to the bath
Carbachol	+ 10
Histamine	- 15
BaCl <sub>2</sub>	+ 17

### Conclusion

Octopirox did not reveal an antagonistic effect against carbachol, histamine, and BaCl<sub>2</sub> in the isolated guinea pig ileum.

## 2.2 Effect of Octopirox on the isolated guinea pig tracheal chain

### Method

A guinea pig trachea was cut spirally at 45° and mounted in O<sub>2</sub> -saturated Ringer's solution at 37° C for isotonic lever recordings. The tracheal strip was contracted with carbachol (final concentration: 10<sup>-7</sup> g/ml). Once a plateau had been reached, tests for a relaxing effect were performed by adding Octopirox and the reference compound isoproterenol in the final concentrations given below. Complete inhibition of carbachol-induced contraction was considered a 100 percent effect.

### Results

Compound	Final concentration	Effect
Isoproterenol	5 x 10 <sup>-7</sup> g/ml	48 % relaxation
Octopirox	10 <sup>-5</sup> g/ml	0% relaxation
	10 <sup>-4</sup> g/ml	0% relaxation

### Conclusion

Octopirox did not have a relaxing effect on the isolated tracheal spiral contracted with carbachol.

3. EFFECT ON CARDIOVASCULAR SYSTEM

### 3.1 Effect of i.v. Octopirox on cardiovascular system in cats

#### Method

Two cats (one male weighing 2.3 kg and one female weighing 2.2 kg) were anesthetized by intraperitoneal injection of pentobarbital sodium 40 mg/kg, and the following parameters were recorded:

1. Arterial blood pressure in the femoral artery
2. Heart rate as integrated from the difference between systolic and diastolic blood pressure
3. Pressure rise in the left ventricle (dp/dt max) as obtained from electronic differentiation of ventricular pressure, which was measured via a PE catheter pushed into the right carotid artery and connected to a Statham P 23 dB pressure transducer
4. Arterial blood flow in the left femoral artery as recorded with an electromagnetic flow meter
5. Respiratory rate and minute volume via back-pressure tube and pneumotachometer.

Amplifiers and recorders were Hellige products. Octopirox was available as a 1 percent aqueous solution and administered intravenously into the right femoral vein.

#### Results

see Table

## 3.1 cont'd 1

Results

Octopirox i. v.	Minutes after injection	Arterial pressure syst./diast. mm Hg	Heart rate beats/min	dp/dt (max) mm Hg/sec	Blood flow ml/min	Respir. rate per min.	Respir. vol. ml/min
Cat I 1 mg/kg	0	150/65	180	6,600	6	2.5	0.2
	1	150/65	180	6,600	6	2.5	0.2
	3	155/65	180	6,700	6	3	0.2
3 mg/kg	0	140/55	184	6,000	6	3	0.2
	1	125/45	184	5,500	6	3	0.2
	3	140/55	180	6,000	6	3	0.2
Cat II 3 mg/kg	0	180/125	188	7,000	5	6	0.2
	1	170/110	188	7,000	5	6	0.2
	3	180/120	190	7,000	5	7	0.3
5 mg/kg	0	180/120	190	7,000	5	7	0.2
	1	145/90	190	6,600	5	7	0.2
	3	175/120	188	7,000	4	7	0.3

### 3.1 cont'd 2

#### Conclusion

Intravenous Octopirox administered in a dose of 1 mg/kg did not have a cardiovascular effect in anesthetized cats. A dose of 3 mg/kg induced a very slight, immediately reversible blood pressure decrease by maximally 15/10 mm Hg. After 5 mg/kg, the blood pressure fell by 35/30 mm Hg, but returned to the initial value within 3 minutes. The remaining cardiovascular and respiratory parameters remained unchanged.

4. EFFECT ON BRONCHIAL SYSTEM

4.1 Effect of i.v. Octopirox on pulmonary function in anesthetized guinea pigs

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Method

Pulmonary overflow was recorded in female guinea pigs (380 g) anesthetized with urethane which were submitted to artificial respiration under constant pressure, using the method after Konzett and Rössler, Naunyn-Schmiedeberg's Arch. exp. Path. Pharmacol. 195, 71 (1940). The animals were pretreated intravenously with Octopirox in doses of 1 and 3 mg/kg or the reference compound isoproterenol in a dose of 0.001 mg/kg. One minute later, experimental bronchoconstriction was induced by intravenous administration of 10 µg histamine dihydrochloride per kg body weight. The degree of bronchoconstriction induced by histamine dihydrochloride was taken as 100 percent. The inhibition of histamine effect in percent (mean value, two experiments) is a measure for the bronchospasmolytic effect.

Results

Treatment	% Inhibition
Octopirox	
1 mg/kg	0
3 mg/kg	0
Isoproterenol	
0.001 mg/kg	92

Conclusion

Intravenous Octopirox administered in doses of 1 and 3 mg/kg did not have a bronchospasmolytic effect in guinea pigs.

5. EFFECT ON METABOLISM

## 5.1 Effect of oral Octopirox on diuresis in rats

### Method

The test was performed after Lipschitz and coworkers, J. Pharmacol. exp. Ther. 79, 97 (1943). The animals were divided into groups of 3 rats and deprived of food and water 24 before the experiment was started. Two groups received then orally 5 mg Octopirox per kg body weight in a volume of 0.5 ml/100 g, and two other groups were given orally the diuretic urea in a dose of 1 g/kg as reference. Subsequently, all animals received a saline load of 5 ml 0.85 percent NaCl solution per 100 g body weight. The urine volume excreted during 5 hours by the test group was compared with that of the reference group.

The Lipschitz value L

$$= T/U = \frac{\text{urine volume of test group (ml/100 g rat/5 h)}}{\text{urine volume of reference group (ml/100 g rat/5 h)}}$$

is a measure for the diuretic activity of a compound. Values of 0.3 - 0.8 indicate the absence of a diuretic effect, values of  $>0.8$  reveal that a compound is diuretically active.

### Results

Treatment	No. of animals	Urine ml/100 g	L = T/U
Urea 1 g/kg	2 x 3	1.59	
Octopirox 5 mg/kg	2 x 3	0.91	0.57

5.1 cont'd 1

Conclusion

Oral Octopirox administered in a dose of 5 mg/kg did not reveal a diuretic effect in rats.

## 5.2 Effect of oral Octopirox on blood glucose in glucose-loaded rats

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### Method

Ten male rats weighing about 250 g were used for the experiment. They were deprived of food 16 hours before the study and then divided into two groups, one of which received orally 5 mg Octopirox per kg body weight in 0.4 percent starch suspension and the other only starch suspension 10 ml/kg. Fifteen minutes afterwards, 1 g glucose per kg body weight was injected subcutaneously as a 50 percent aqueous solution to the animals of both groups. Blood samples were taken from the tail vein immediately before treatment as well as 1, 3, and 5 hours afterwards. Blood glucose was determined after the hexokinase method (Böhringer test combination).

### Result

Treatment	Blood glucose		
	mMol 0 hour	% Change 1 hour	% Change 3 hours
Octopirox 5 mg/kg	3.58	+ 93	+ 13
Control	3.51	+ 98	+ 9

### Conclusion

Oral Octopirox in a dose of 5 mg/kg did not have an effect on blood glucose in glucose-loaded rats.

5.3 Effect of oral Octopirox on carrageenan-induced paw edema  
in rats

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Introduction

The injection of carrageenan into the hind paw of rats induces an inflammatory edematous swelling which is measured volumetrically at certain time intervals. Pretreatment with anti-inflammatory agents inhibits the increase in swelling in dependence of the dose.

Method

The study was performed after the method of Ch. Winter and co-workers, Proc. Soc. exp. Biol. (N.Y.) 111, 544 (1962). Groups of 5 male Sprague Dawley rats, which had been fasted 17 hours before the experiment, were pretreated orally with 5 mg Octopirox per kg body weight, 80 mg phenylbutazone per kg body weight, or the vehicle - a one percent methylcellulose suspension in a volume of 10 ml/kg. Thirty minutes afterwards the animals received 0.05 ml of a 0.5 percent carrageenan solution in physiological saline supplantar into the left hind paw.

Results

Treatment	Init. volume in ml, $\bar{x}$	Swelling increase in ml, $\bar{x}$	
		after 3 hrs.	after 6 hrs.
Octopirox 5 mg/kg	1.29	0.30	0.32
Phenylbutazone 80 mg/kg	1.33	0.14	0.18
Control	1.33	0.22	0.21

### 5.3 cont'd 1

#### Conclusion

Oral Octopirox administered in a dose of 5 mg/kg did not exhibit anti-inflammatory activity in the carrageenan-induced paw edema in rats.

#### 5.4 Effect of oral Octopirox on body temperature in febrile rats

##### Method

Groups of 5 male Sprague Dawley rats (Gassner, 180 - 225 g) per dose were used. They received a subcutaneous injection of 15 percent brewer's yeast in a volume of 1 ml/100 g body weight to produce a febrile state. After the injection, the animals were deprived of food for 17 hours. In the 18th hour, the body temperature was measured rectally twice at 15-min. intervals to obtain the febrile initial temperature. Subsequently, the rats were given orally 5 mg Octopirox per kg body weight, 40 mg aminopyrine per kg body weight, or the vehicle as reference - a 1 percent methylcellulose suspension in a volume of 10 ml/kg. The body temperature was measured 30, 60, 120, and 240 minutes after administration of the test compounds.

##### Results

Treatment	Init. temp. °C, $\bar{x}$	Change against initial value			
		30 min	60 min	120 min	240 min
Octopirox 5 mg/kg	38.4	+ 0.2	- 0.1	- 0.4	- 0.3
Aminopyrine 40 mg/kg	38.3	- 1.2	- 1.3	- 1.1	- 0.9
Control	38.5	+ 0.1	± 0.0	- 0.1	- 0.1

##### Conclusion

Oral Octopirox administered in a dose of 5 mg/kg did neither reduce nor increase the body weight in febrile rats.