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Anticonvulsant and sedative effects of some 5-substituted bromopyrazolinic spirobarbiturates☆

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Abstract

We studied in mice the neuropharmacological effect of some 5-substituted bromopyrazolinic spirobarbiturates. LD_{50} and CL_{50} values were estimated for all the investigated compounds. The effect on potentiation of sodium pentobarbital activity and on generalised tonic–clonic seizures produced by PTZ on mice was studied. All the compounds had the same pharmacological profile, they extended sleeping-time induced by sodium pentobarbital and showed protection against PTZ-induced convulsions, decreasing the death rate. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: Spirobarbiturates; LD_{50} and CL_{50}; Irwin test; Anticonvulsant and sedative effects

1. Introduction

Numerous derivatives of barbituric acid had been widely used as anxiolytic and sedative-hypnotic agents before the advent of the safer benzodiazepines. Currently, the clinical applications of the barbiturates are limited to the treatment of certain epilepsies and as intravenous anaesthetics. Use of barbiturates was associated with drug abuse, drug dependency and pharmacokinetic and pharmacodynamic tolerance.

Spirobarbiturates, characterised by the Br-pyrazolinic ring on C_5 of barbituric acid, are new compounds never tested as central nervous system (CNS) depressant agents. In this work, we report the sedative and anticonvulsant activity of these compounds in the mouse.

2. Experimental

The compounds tested in this study (Fig. 1) were synthesised according to the methods reported elsewhere [1,2]. They differ in the *para*-substituent on the benzene ring (**a**, H; **b**, OCH_3; **c**, Cl; **d**, NO_2). This synthesis produced regioisomers, which are compounds presenting a single bond, 5–5′ and 5–4′, between barbituric acid and the bromopyrazolinic substituent.

The regioisomers were subjected to column chromatography, but this technique was not able to separate the isomers **4c**/**4′c**. The quantity of the regioisomer **4d** obtained was not sufficient for the biological trials. All investigated compounds were dissolved in propylene glycol and administered intraperitoneally (i.p.) to the mice.

2.1. Animals

Swiss mice (30–35 g) were used in the experiments. They were kept in standardised conditions (temperature 22 ± 2°C, humidity 60 ± 4%, natural lighting), fed with a standard diet (S. Morini, Mill rat GLP) and water ad libitum. In all experiments the mice were divided in seven groups of ten animals each. The first was the control group.

Data were expressed as mean $\pm$ SE of ten determinations. The results were statistically analysed by Student's *t*-test; $P < 0.05$, versus control was taken as significant.

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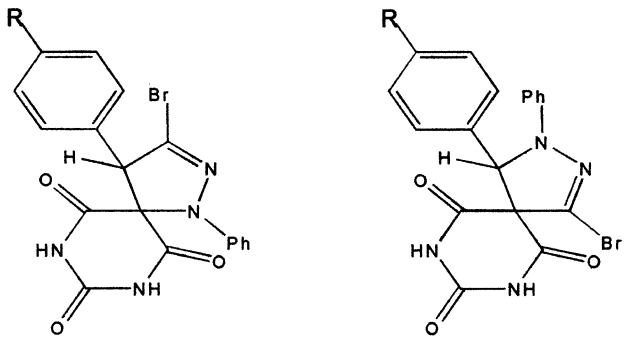
2.2. Acute toxicity

Different doses of the compounds were administered i.p. to the mice fasted for 18 h. Then these animals had been allowed access to food and water ad libitum and were observed over a period of seven days to monitor clinical signs and mortality [3].

2.3. LC_{50} determination

LC_{50} determination was carried out by the method of Meyer [4], utilizing brine shrimps (*Artemia salina* L.). This is a bioassay system largely employed in LC_{50} determination. Compound activity is manifested as toxicity. Activity of the compounds is manifested as toxicity to the shrimp. Appropriate amounts of compounds (10, 100 and 1000 μg/ml) were assayed. LC_{50} was determined from 24 h counts using the probit analysis [5].



4 (5-5') 4' (5-4')

Compounds	R	M.p. (°C)
4a	H	157
4'a	H	173
4b	OCH_3	128
4'b	OCH_3	168
4'd	NO_2	192
4c/4'c	Cl	—

Fig. 1. Chemical structures of spirobarbiturates.

Table 1
LD_{50} and CL_{50} of the compounds

Comp.	LD_{50} (mg/kg)	CL_{50} (μg/ml)
4′a	150	44.95
4a	200	1000
4′b	150	47.07
4b	200	629.03
4′d	150	115
4c/4′c	200	65.35

2.4. Irwin test

The Irwin test [6] is a scanning procedure designed to assess and characterise the effects of a drug, in particular, on the behavioural sphere in the rat and mouse. The effects of all tested compounds were observed after i.p. administration at a dose of 25 and 50 mg/kg. The animals were monitored for seven days and were also observed for their behavioural symptoms.

2.5. Potentiation of hypnotic effect of sodium pentobarbital

According to the method described by Carlini [7], the control group was given the vehicle only, while the other groups were treated with the different compounds (25 mg/kg i.p.). Thirty minutes later, all mice were administered with sodium pentobarbital (50 mg/kg i.p.) in physiological saline. Induction time (time elapsed between injection of sodium pentobarbital and loss of the righting reflex) and sleeping time (time interval between loss and recuperation of the righting reflex) were recorded. The criterion for recuperation of the righting reflex was fixed so that the mice had to regain their normal posture three consecutive times.

2.6. Chronic treatment

The investigated compounds were administered at the dose of 50 mg/kg for ten consecutive days. Induction time and sleeping time were recorded.

2.7. Anticonvulsant activity

The anticonvulsant activity of these compounds was demonstrated on generalised tonic–clonic seizures induced by pentylenetetrazol (PTZ) in mice [8]. The control group was treated with the vehicle, while the other groups were injected with the investigated compounds (25 mg/kg i.p.). Thirty minutes later, all mice received PTZ (90 mg/kg i.p.). Animals were observed for 1 h to monitor the period of latency (time interval between PTZ administration and beginning of convulsion), the number and duration of the convulsions and to note lethality.

3. Results and discussion

LD_{50} values for the investigated compounds after i.p. administration in mice are presented in Table 1. The values indicate that LD_{50} was between 150 and 200 mg/kg.

Table 2
Effects of the compounds on sodium pentobarbital induction time and sleeping-time potentiation in mice

Comp.	Dose (mg/kg) (i.p.)	Induction time X (min $\pm$ SE)	Sleeping time X (min $\pm$ SE)
4′a	25	2 ± 0.8 *	52 ± 3.5 *
4a	25	4 ± 1.2	58 ± 3.9 *
4′b	25	2 ± 0.7 *	46 ± 2.5 *
4b	25	2 ± 0.6 *	100 ± 4.9 *
4′d	25	5 ± 1.0	54 ± 4.0 *
4c/4′c	25	2 ± 0.5 *	98 ± 4.2 *
CTR		5 ± 0.9	38 ± 2.7

* $P < 0.05$ compared with control.

Table 3
Effect of the compounds on PTZ-induced seizures in mice

Comp.	Dose (mg/kg) (i.p.)	No. of seizures $X \pm$ SE in 10 min	Mortality (%)
4′a	25	0.50 ± 0.37 *	20
4a	25	0.16 ± 0.12 *	20
4′b	25	1 ± 0.67	20
4b	25	0.33 ± 0.23 *	10
4′d	25	0.66 ± 0.42 *	20
4c/4′c	25	0.50 ± 0.35 *	20
CTR		3 ± 0.64	80

* $P < 0.05$ compared with control.

CL_{50} values for the investigated compounds are between 44.95 and 1000 μg/ml (Table 1).

Intraperitoneal administration of all compounds determined in mice a less sensitive reaction to external stimuli. In fact after 20 min, the dose of 25 mg/kg produced stereotyped movements, passivity, loss of curiosity. These phenomena were remarkable at the dose of 50 mg/kg; so the response was dose-dependent. Twenty-four hours after treatment the animals came back to normality.

The administration of all tested compounds increased the hypnotic effects of sodium pentobarbital; decrease of the induction time together with the enhancement of the sleeping time were observed. Induction time was of 5 min in mice treated only with pentobarbital, while in animals which received the compound **4c**/**4′c** it was significantly decreased to 2 min. The sleeping time goes from 30 min in controls to 88 min in mice treated with compound **4b** (Table 2). During the chronic treatment, the investigated compounds showed the same values of induction and sleeping times.

The investigated compounds were able to protect against PTZ-induced convulsions, reducing their duration and number, increasing the period of latency and decreasing the mortality. The control group, treated with PTZ only, showed four convulsions in a range of 30 min. Moreover, after 20 min 80% mortality was observed. The mice treated with the tested compounds showed one convulsion in a range of 30 min after PTZ injection, and mortality was 25% (Table 3).

From the result, all the tested compounds seem to have the same pharmacological profile, so the different substitutions on the benzene ring do not modify the activity. The comparison between the activities of the investigated regioisomers did not show significant differences.

A particularly interesting result was the absence of tolerance in the mice during chronic treatment. Probably these compounds, unlike the well-known barbiturates, do not cause enzymatic induction and do not modify CNS sensibility.

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