

PHARMACODYNAMIC STUDY OF AROMATIC AND HETEROCYCLIC DIETHYLAMIDES *

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SUMMARY: The analeptic effect of Anacardiol compared with that of Nikethamide and Pentylenetetrazol has been studied in rats, cats, dogs, and rabbits. Anacardiol had an irregular effect, inducing in some cases abnormal respiratory patterns, including apnea. Pentylenetetrazol had a clear cut effect, and Nikethamide was mildly effective. However, its depressor component limits its use. Comparing the reported LD₅₀ in rats and our experimental effective doses for Nikethamide, Pentylenetetrazol, and Anacardiol, the last one would possess the smallest safety ratio.

DESCRIPTORS: diethylamides, aromatic and heterocyclic; diethylamides, pharmacodynamics.

INTRODUCTION

The rational introduction of an active substance in medicine requires a formal assessment of its therapeutic merit, compared with those of existing drugs, when these exist.

This criterion, and the controversial use of analeptics in medicine has prompted us to evaluate the pharmacological properties of one of them, Anacardiol, comparing it with Nikethamide, and Pentylenetetrazol (fig 1).

"The term analeptic usually refers to a drug able to restore depressed medullary and other functions of the central nervous systems (CNS). This definition is applicable to drugs of very different pharmacological groups. It includes not only substances which act by primary excitation of the CNS, but also substances which act by competition with depressants, or by opposing metabolic or circulatory disturbances in the CNS. From a pharmacological standpoint it is advisable to

restrict the term Analeptic to substances which stimulate the normal as well as the depressed CNS, presumably by the same elementary mechanism".

It is generally accepted that the analeptic activity of alkylated amides is due to the alkylation of the nitrogen of the amide group.

The study of the respiratory actions of these analeptics has provided some interesting information about their structure-activity relationship. HOFFER & REINERT¹⁰ found that among the derivatives of the aromatic and heterocyclic amides, complete substitution of the amide nitrogen with ethyl groups provides optimal activity.

Many derivatives of benzoic-acid-diethylamide have been prepared, and the following substances have been found to be the most potent as to analeptic activity: 4-hydroxy-3-methoxy-benzoic acid-diethylamide (Ethamivan), and 4-hydroxy-3-ethoxy-benzoic acid-diethylamide (Anacardiol).

* Work done at the Instituto Nacional de Farmacología y Bromatología, Buenos Aires, Argentina.

** From the Instituto Nacional de Farmacología y Bromatología.

Modification of the ring of the acid component of the molecule led to various substances of which pyridine-3-carboxylic acid-diethylamide (Nikethamide) is the most important one (fig. 1).

MATERIAL AND METHODS

The following drugs were used: Anacardiol, powder (Laboratório Farmacêutico Dupomar, Buenos Aires, Argentina); Nikethamide, Co-ramina[®] CIBA, ampoules containing 0.25 g/ml in distilled water; Pentylenetetrazol, Knoll[®], Pharm. Helv. V, DAB[®], Chemische Fabriken, Switzerland. Vehicle, sodium chloride solution.

Balb/c Mice (18-22 g), Wistar Rats (80-200 g), Dutch Belted Rabbits (2.5-3.0 kg) outbred from the I.N.F. y B. colonies, mongrel Cats (3.0-3.5 kg), and Dogs (7.0-10.0 kg) of either sex, were used. They were fasted overnight, and then anesthetized with 40 mg/kg of pentobarbital sodium given intraperitoneally. The drugs were injected through a cannula in the right femoral vein. Heparin

(132 U.I./mg) was used as an anticoagulant (1,000 U.I./kg).

The LD₅₀ of Anacardiol was determined using 200 mice, and 50 rats; 10 mice, and 10 rats per dose-level, and five doses for assay logarithmically spaced. The animal were observed during seven days. A control group was injected with the vehicle. The data were evaluated following the Bliss Statistical Method⁴.

Blood pressure in 10 intact rats was measured by the Agrelo, Dawson Method².

Determinations of the stimulant action on respiration, blood pressure, and E.C.G. in 10 rats, 8 cats, 2 dogs, and 2 rabbits were carried out using a Statham P23 AC, via a catheter in the left carotid artery, for blood pressure; with a Grass volumetric pressure transducer Pt 5 for respiration; and three suitable electrodes for E.C.G. Transducer signals were recorded on a Grass Model 7 Polygraph.

Similar experiments were conducted in a group of animals depressed with morphine (4 rats, 2 rabbits, 2 cats, 2 dogs), and in 4 cats pretreated with reserpine.

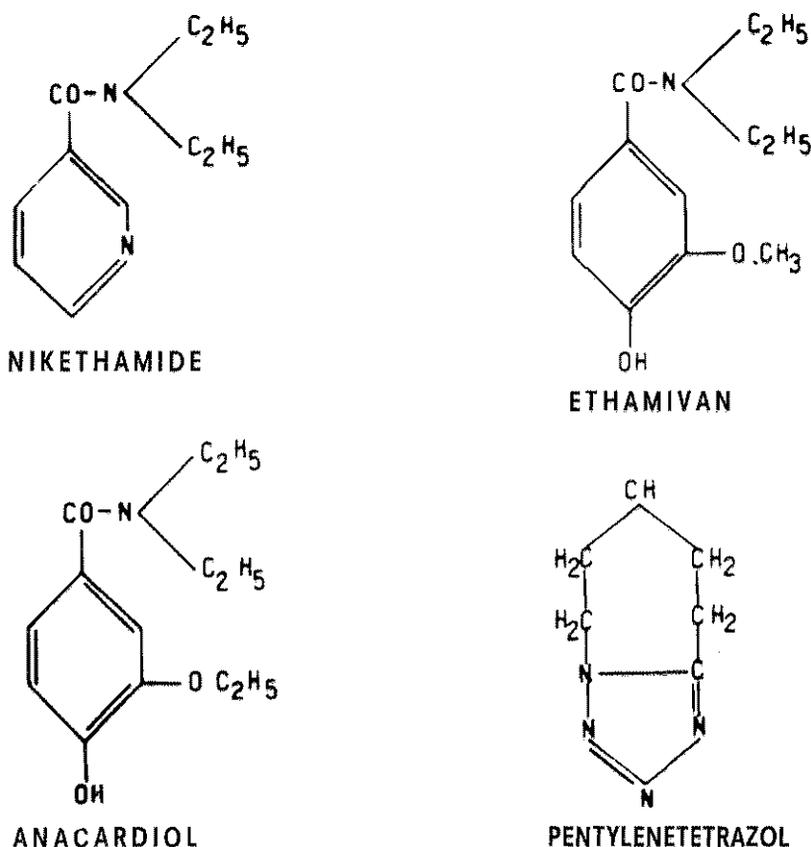


Fig. 1 — Chemical structures of the evaluated analeptics.

RESULTS

Acute toxicity — Anacardiol mice LD₅₀ I.V. = 5.4 mg/kg ± 0.5. Observations: for lower doses the common symptoms were increased motor activity; for intermediate doses, tremors and twitches; and for higher doses, tonic clonic convulsions. Rat LD₅₀ I.V. = 6.9 mg/kg ± 0.3. Similar symptoms were observed.

1. Effect on blood pressure on unanesthetized animals

The effect of Anacardiol, Nikethamide, and Pentylenetetrazol on blood pressure was studied on each one of ten intact unanesthetized rats. Effects on blood pressure with Anacardiol doses of 1, and 2 mg/kg were not observed. 2.5 mg/kg caused clonic convulsions. On the same animals, once recovered, administration of 20 mg/kg of Pentylenetetrazol elicited persistent twitches without alterations of the blood pressure.

Nikethamide (40 mg/kg) produced a transient fall in blood pressure with immediate recovery.

2. Effect on animals under anesthesia by barbiturates

Cardiovascular system — In anesthetized animals (rats, cats, dogs), depressed with barbiturates, after administration of different doses of Anacardiol, an initial fall in blood pressure was observed returning to initial or lower levels. In rabbits, Anacardiol was

mainly pressor as it is shown in table 1. Nikethamide tends to produce vasodepressor effects, and Pentylenetetrazol showed pressor effects on blood pressure. There were no significative changes observed in any of the registered E.C.G.

Respiratory function — In anesthetized rats, respiratory amplitude was enhanced with 2 to 10 mg/kg of Anacardiol, but the response is irregular and transient, lasting only 2 min. (fig. 2). The reference drugs, Nikethamide (40 mg/kg), and Pentylenetetrazol (10 and 20 mg/kg) had a slightly greater response on respiratory amplitude, and it did last longer, approximately 8 min, and it was reproducible (fig. 3).

In table 2 results with cats are shown.

In dogs under identical conditions, 1 and 2.5 mg/kg of Anacardiol produced no effect, but 5 mg/kg induced apnea. In another experiment, 2 mg/kg induced also a transient apnea (fig. 4). Nikethamide (34 mg/kg), as shown in fig. 5, and Pentylenetetrazol (10 mg/kg) increased respiratory amplitude and frequency.

In anesthetized rabbits (table 1), Anacardiol (2.5 mg/kg) improved the respiration but this response was not reproducible. Under deeper anesthesia with barbiturates, 2.5 mg/kg, and 5 mg/kg of Anacardiol diminished the respiratory function, and 10 mg/kg caused an initial decrease of respiratory amplitude followed by a transient moderate increase. After recovery of the base line, treatment with Pentylenetetrazol (10 mg/kg) had a real stimulant action on blood pressure and respiration.

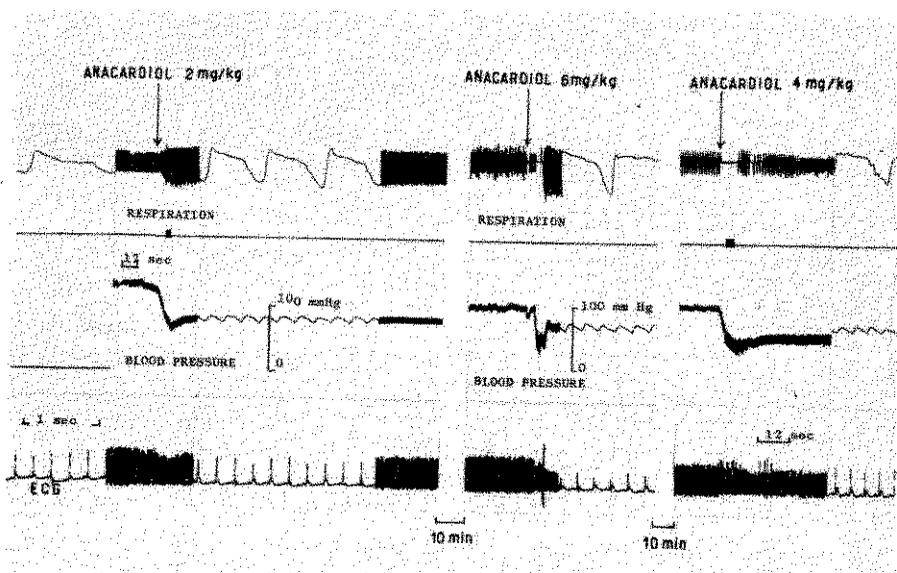


Fig. 2 — Effect on respiration, blood pressure and E.C.G. of Anacardiol, in the rat anesthetized with pentobarbital sodium.

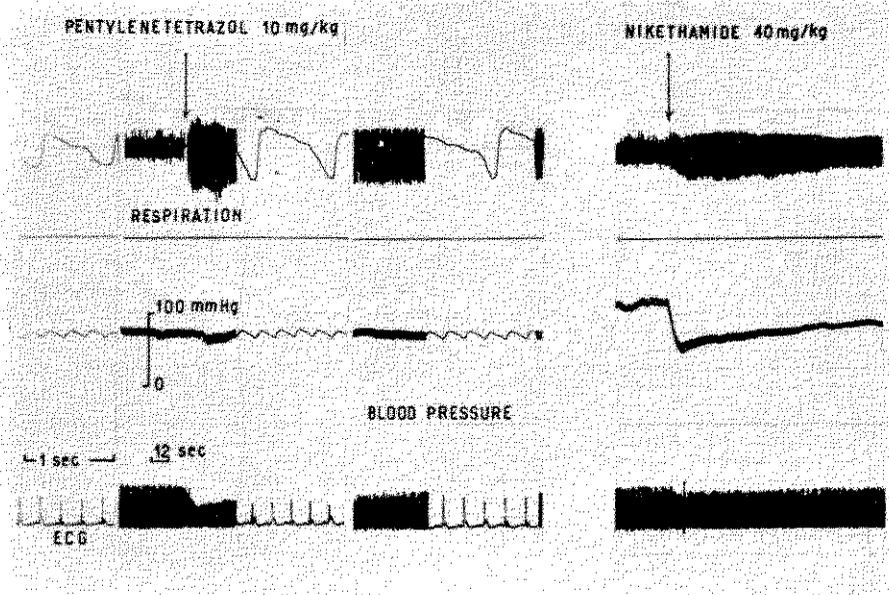


Fig. 3 — Effect on respiration, blood pressure and E.C.G. of Pentylenetetrazol, and Nikethamide in the rat anesthetized with pentobarbital sodium.

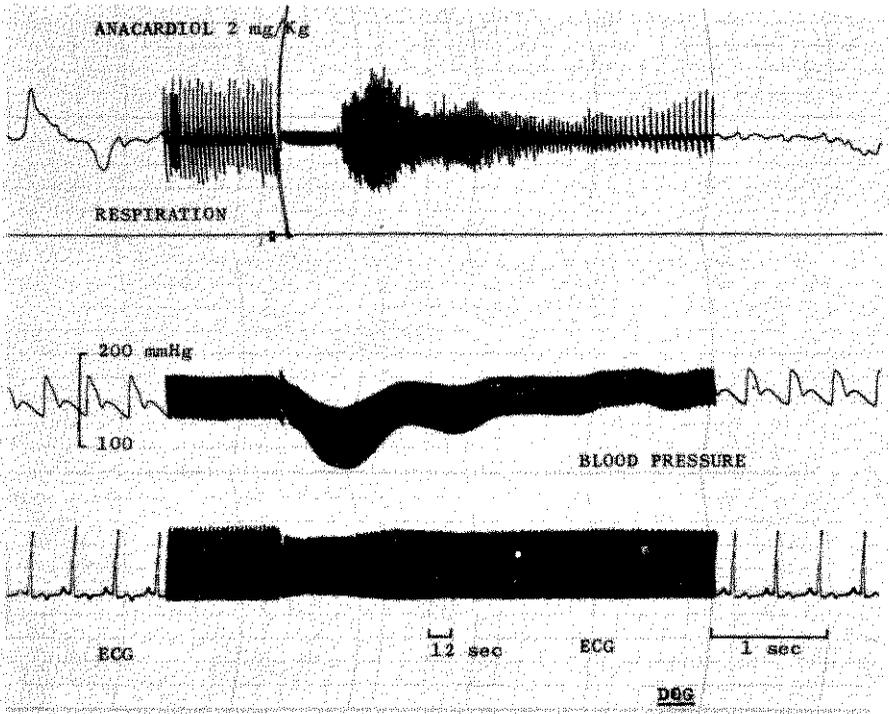


Fig. 4 — Effect on respiration, blood pressure and E.C.G. of Anacardiol in dogs anesthetized with pentobarbital sodium.

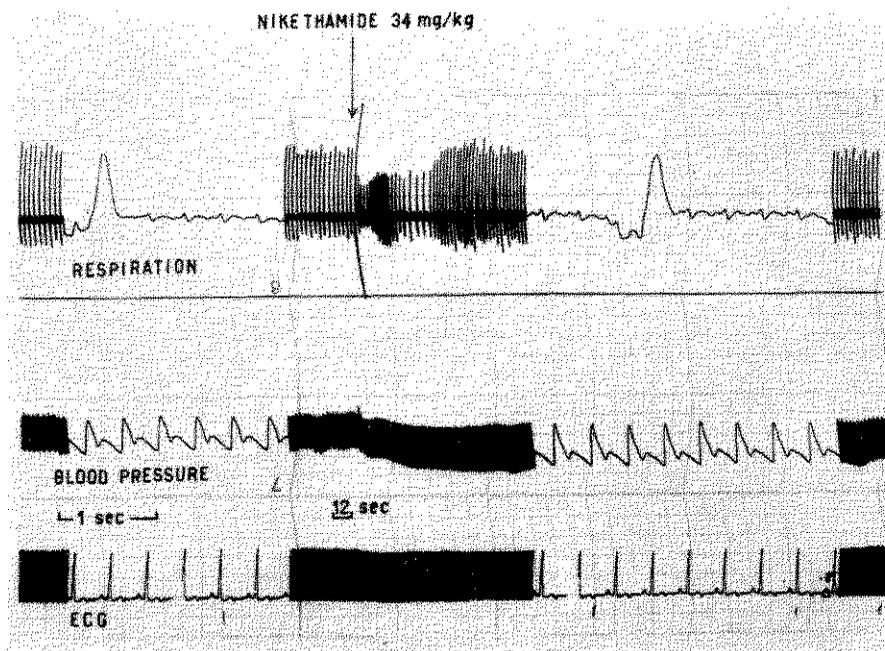


Fig. 5 — Effect on respiration, blood pressure and E.C.G. of Nikethamide in dogs anesthetized with pentobarbital sodium.

TABLE 1

Effects on rabbits under anesthesia by barbiturates

Drug	Doses mg/kg	Effect on respiration	Effect on blood pressure
	2.5	Weak increase of amplitude, without frequency variation. The effect was not a constant one and disappeared with increasing doses.	Increase of 30 mmHg, lasting 4 min.
Anacardiol	5.0	Weak increase of amplitude and frequency.	Initial decrease of 25 mmHg with later stabilization at 15 mmHg, higher than basal levels.
	10.0	Initial decrease of amplitude followed by a moderate increase, lasting 6 min.	Initial decrease of 30 mmHg lasting 1 min with later stabilization at 15 mmHg, higher than basal levels.
Pentylentetrazol	10.0	Marked increase of frequency and moderate increase of amplitude.	Increase of 30 mmHg lasting 8 min.

TABLE 2

Effects on cats under anesthesia by barbiturates

Drug	Doses mg/kg	Effect on respiration	Effect on blood pressure
Anacardiol	1.0	No effect.	No effect.
	3.0 4.0	Immediate after injection occurred a marked decrease of amplitude, and a moderate increase of frequency. After 1 min, the situation was normalized to the initial frequency but of lower amplitude.	Initial decrease with return to normal after 3 min (initial value).
	10.0	Weak increase of amplitude, and decrease of frequency.	—
	20.0	Convulsions.	Convulsions.
Nikethamide	56.0	Initially it decreased the amplitude and increased the frequency with restoration of basal levels after 3 min.	Decrease of 45 mmHg returning to normal values after 5 min.
	70.0	As with 56 mg/kg.	Decrease of 60 mmHg returning to normal values after 8 min.
Pentylentetrazol	5.0	No effect.	No effect.
	10.0	Marked increase of amplitude and frequency lasting 10 min.	Increase of 50 mmHg returning to normal values after 10 min.

3. *Effects on anesthetized animals depressed with morphine*

In rats, anesthetized with pentobarbital and depressed with morphine, respiration was relatively enhanced with 2, 6 and 10 mg/kg of Anacardiol, while the last dose under a very deep anesthesia, showed to cause an occasional apnea. With Nikethamide (62 mg/kg) the enhancement of the respiratory function was much greater and reproducible, and 10 and 20 mg/kg of Pentylentetrazol also induced a marked effect on respiration amplitude and frequency.

In anesthetized cats, 10 mg/kg of Anacardiol increased, under the influence of morphine, the depression of the respiratory function leading to apnea. The animal had to be recovered with Pentylentetrazol (20 mg/kg).

With dogs depressed with morphine (table 3), injections of Anacardiol (from 1.5 to 5 mg/kg) developed a Biot's breathing (fig. 6) With higher doses there was an enhancement of these respiratory patterns. In a number of cases it was necessary to use Pentylentetrazol to recover the animal. Nikethamide (34 mg/kg) also restored the previous respiratory frequency.

Results with rabbits are shown in table 4. It can be seen that 10 mg/kg of Anacardiol induce apnea simultaneously with a pronounced blood pressure fall. Pentylentetrazol (20 mg/kg) was used to recover the initial respiratory rhythm and blood pressure (fig. 7).

Pretreatment of cats, 16 hours prior to the assay with a single intraperitoneal injection of 3 mg/kg of reserpine, did not change the pattern of responses obtained in anesthetized animals without reserpine.

TABLE 3

Effects on dogs anesthetized, having its respiratory function depressed with morphine

Drug	Doses mg/kg	Effect on respiration	Effect on blood pressure
Anacardiol	1.5	Very weak initial decrease of amplitude with modifications of rhythm according to Biot's type.	No effect.
	2.5	Biot's breathing.	Weak initial decrease with imm. normalization.
	5.0	As before but much more marked.	Initial moder. decrease with imm. normalization.
Nikethamide	34.0	Marked increase of frequency.	Weak decrease.
Pentylenetetrazol	10.0 to 25.0	Marked increase of amplitude and frequency.	No effect.

TABLE 4

Effects on rabbits having its respiratory function depressed with morphine

Drug	Doses mg/kg	Effect on respiration	Effect on blood pressure
Anacardiol	2.5	No effect.	Initial weak decrease with immediate recovery.
	5.0	Initial decrease of amplitude and frequency, increasing later only its amplitude.	Initial weak decrease with immediate recovery.
	10.0	Respiratory arrest.	Marked decrease of 45 mmHg.
Pentylenetetrazol	20.0	Recovery of the animal after respiratory arrest.	Recovery and increase to 25 mmHg above basal level lasting 8 min.

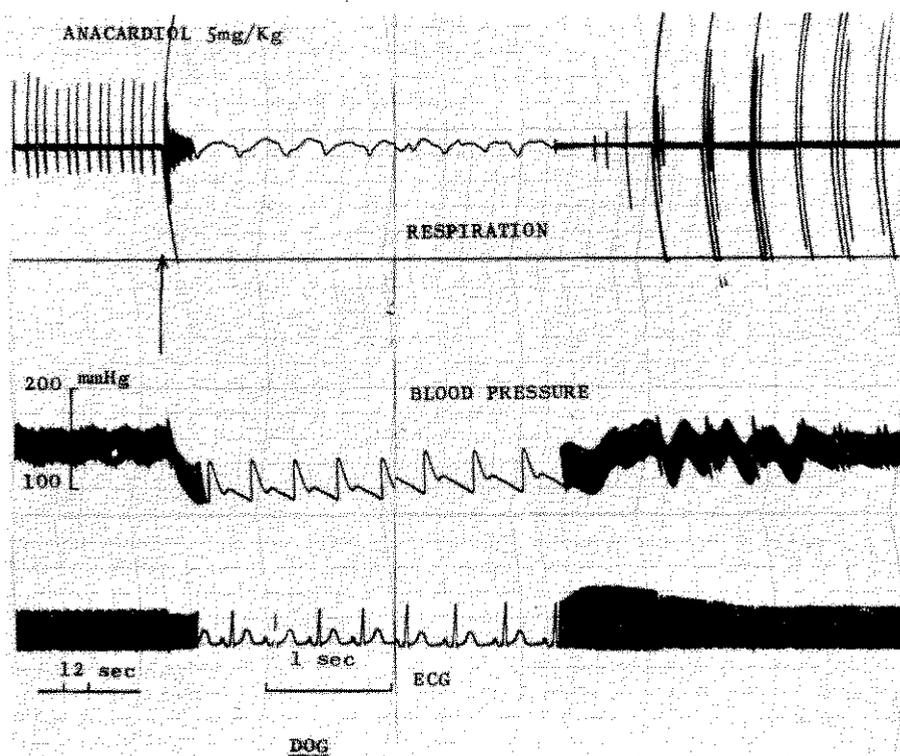


Fig. 6 — Effect on respiration, blood pressure and E.C.G. of Anacardiol in dogs anesthetized and depressed with morphine.

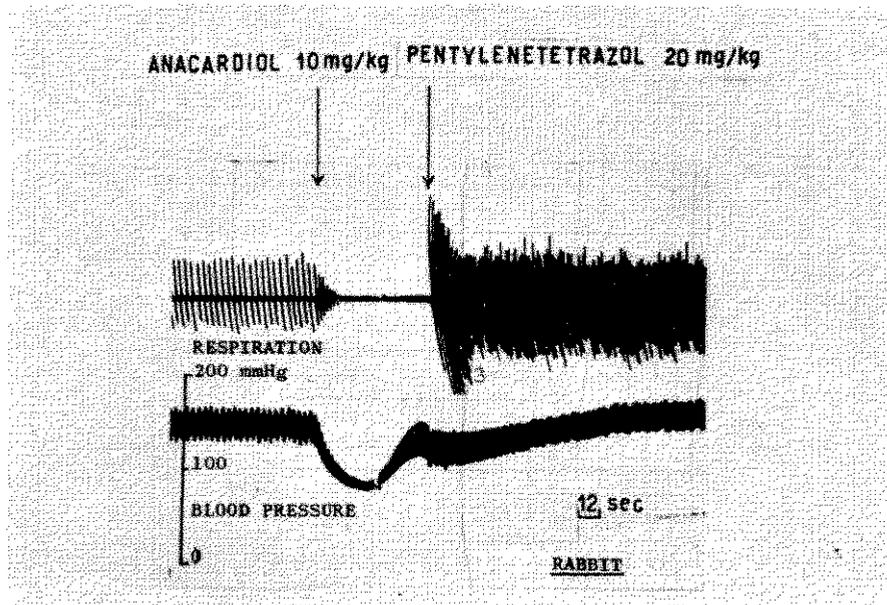


Fig. 7 — Effect on respiration and blood pressure of Anacardiol, and Pentylenetetrazol in anesthetized rabbits depressed with morphine.

DISCUSSION

Strong objection have been raised to the use of analeptics in the treatment of CNS depression, and supportive treatment without analeptics has been recommended. It could be said that the only field of therapeutic use of these drugs is that of depression of the CNS by anesthetics, specially barbiturates⁶.

ADRIANI *et alii*¹ stated that narcotized patients with I.V. barbiturates could be recovered by analeptics with the following order of efficacy, Bemegrade, Ethamivan, Picrotoxin, Pentylenetetrazol, and Nikethamide. Picrotoxine, Bemegrade, and Pentylenetetrazol are used in such cases because of their effect on respiration and circulation, and their general arousal effects. Picrotoxin possessed the smallest therapeutic range.

An important difference between Nikethamide and Pentylenetetrazol is that the former has a component of central depression. This effect limits the usefulness of Nikethamide in the treatment of barbiturates poisoning because it may add its action to the ones of barbiturates prolonging anesthesia and increasing mortality. Anacardiol, like Nikethamide, sometimes prolonged the anesthesia in humans too, instead of facilitating wakefulness⁷.

Ethamivan is a compound related to Anacardiol and Nikethamide in its chemical structure, and its pharmacological profile follows closely that of Nikethamide. It has been reported that I.V. administration of Ethamivan to animals is often followed by a transient period of apnea after which the respiration becomes fast and deep⁸.

Substitution of the ethyl group of Anacardiol for the methyl group of Ethamivan produces an increase of the central depressor effect observed with the structurally related Nikethamide. Similar replacement of the methyl group of Ethamivan by bromine or chlorine produced a decrease in the analeptic effect⁹.

BOTTA⁵ found that Anacardiol caused an initial fall of blood pressure that could be due to a splanchnic vasodilatation with further return to higher levels. Our experimental results are generally not in accordance with that, except with rabbits. Even though it was observed an initial blood pressure fall, it returned to basal or lower levels. Nikethamide tended to produce vasodepressor effects too, in agreement with DAS & CHOWDHURI⁸. Pentylenetetrazol showed pressor effects on blood pressure.

In our experiments, animals depressed with barbiturates, or with barbiturates plus morphine, Pentylenetetrazol showed better effects than Nikethamide, and Anacardiol on the improvement of the respiratory function, while responses to Anacardiol were not reproducible.

In anesthetized dogs we also observed with Anacardiol the transient period of apnea reported for Ethamivan⁸, followed in our case by an increase of frequency during a short time (2 min), and later stabilization to lower amplitudes and rythm than those previous to drug administration.

In anesthetized rabbits which were previously treated with morphine, apnea was also observed although much more prolonged, Pentylenetetrazol being necessary to recover the animals.

During our experiments, with dogs anesthetized and depressed with morphine, Anacardiol induced Biot's breathing. Periodicity of this type is encountered particularly in disease or injury involving the brain itself.

Anacardiol would have an irregular effect inducing, in some cases, abnormal respiratory patterns including apnea, also producing an increase of the central depressor effect, and the reported prolongation of anesthesia in humans⁷. These actions, in relation to those of Ethamivan and the related Nikethamide, show once again that small modifications of the chemical structure may raise important pharmacological changes.

Taking into account the LD₅₀ in rats for Nikethamide, and Pentylenetetrazol reported by BARNES & ELTHERINGTON⁶; the effective cardiorespiratory doses for the rat (40 mg/kg for Nikethamide, 15 mg/kg for Pentylenetetrazol, and 8.5 mg/kg for Anacardiol), calculated from usual human single effective doses; and our experimental effective ones, Anacardiol would have the smallest safety ratio (1.5, Nikethamide 6.0, and Pentylenetetrazol 3.5).

Comparing the pharmacological activity of these compounds in several animal species, our results showed that Pentylenetetrazol and Nikethamide are more active and safer than Anacardiol.

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RESUMO: O efeito analéptico do Anacardiol comparado com o da Niketamida e o do Pentilenetetrazol, foi estudado em ratos, gatos, cães e coelhos. O efeito respiratório induzido pelo Anacardiol mostrou-se irregular, com o aparecimento de quadros respiratórios anormais, inclusive apnéia. O efeito do Pentilenetetrazol foi nítido ao passo que o da Niketamida foi discreto, embora seu componente depressor limite seu uso. O estudo comparativo das DL_{50} publicado para ratos com as nossas doses efetivas experimentais para a Niketamida, Pentilenetetrazol e Anacardiol mostra que este último tem o menor índice de segurança.

DESCRIPTORIOS: dietilamidas aromáticas e heterocíclicas; dietilamidas, farmacodinâmica.

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