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European Journal of Pharmacology 283 (1995) 103–106

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Effect of diterpenoid alkaloids on cardiac sympathetic efferent and vagal afferent nerve activity

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Received 25 April 1995; accepted 9 May 1995

Abstract

The diterpenoid alkaloid, lappaconitine, at a dose of 150 μg/kg (i.v.) increased cardiac vagal afferent nerve activity (16.2%) and reduced cardiac sympathetic efferent nerve activity (12.5%). A polar analog, *N*-deacetyllappaconitine, at this same dose, increased cardiac vagal afferent nerve activity (40%) and reduced cardiac sympathetic efferent nerve activity (23.5%). Both of these agents also reduced arterial blood pressure and heart rate. A larger dose of lappaconitine (300 μg/kg i.v.) produced the same changes in nerve activities and cardiac function as the lower dose. Two other structurally related agents, lycoctonine and aconine, failed to alter these variables in doses up to 300 μg/kg. These data suggest that certain diterpenoid alkaloids activate autonomic reflex receptors, including cardiac reflex receptors. The polar agent, *N*-deacetyllappaconitine, appears to be more effective on cardiac reflex receptors than the non-polar agent, lappaconitine. Such agents may be useful in the treatment of hypertension.

Keywords: Diterpenoid alkaloid; Autonomic nerve; Blood pressure; Heart rate

1. Introduction

Throughout the body, a number of reflexogenic receptor areas which respond to stretch or pressure govern the autonomic nervous tone and affect cardiovascular function. A variety of natural products, including digitalis, are known to activate or sensitize both arterial baroreceptors and cardiac mechnoreceptors, causing (1) an increase in vagal efferent nervous tone and/or (2) a withdrawal of sympathetic efferent nervous activity (Gillis and Quest, 1986). These agents all increase intracellular cation concentration – by different and poorly understood means – to enhance sensory receptor cell firing. The results are a decrease in heart rate and blood pressure.

Polar aminocardenolides have been shown to act selectively on cardiac reflex receptors rather than arterial baroreceptors. These agents increase cardiac vagal afferent nerve activity and reflexly decrease cardiac sympathetic nerve activity (Rebagay and Caldwell, 1990, 1992). Non-polar digitalis agents, like digoxin, act upon both arterial and cardiac reflex receptors (Shepard and Abboud, 1983; Rebagay and Caldwell, 1990, 1992; Caldwell and Rebagay, 1992).

Certain diterpenoid alkaloids isolated from plant species *Aconitum, Delphinium* and *Consolida* have hypotensive and bradycardiac actions which may be due to activation of autonomic reflexes (review by Pelletier, 1983). One agent, lappaconitine, has been shown to reduce blood pressure and heart rate and possess a low toxicity (Benn and Jacyno, 1983). The goal of this study was to determine if lappaconitine and *N*-deacetyllappaconitine, a much more polar derivative, work through activation of autonomic reflex receptors. Other agents of this chemical class were also examined.

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Supported by NIH grant HL-32562.

Elsevier Science B.V.
SSDI 0014-2999(95)00290-1

2. Materials and methods

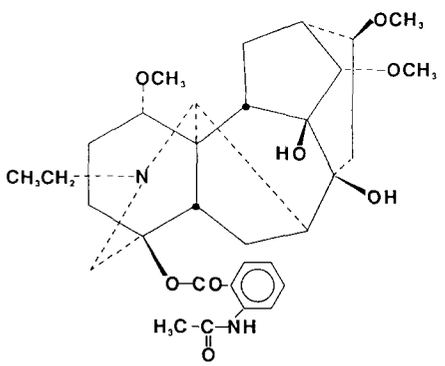
2.1. Materials

Lappaconitine was isolated and *N*-deacetyllappaconitine was synthesized in the laboratory of one of us (S.W.P.). The structures for these compounds are given in Fig. 1. Lycoctonine and aconine were also synthesized by S.W.P.'s research group. Each crystalline material was dissolved fresh daily in saline for experiments. Morphine sulfate and sodium pentobarbital were obtained from Sigma Chemical Company (St. Louis, MO, USA).

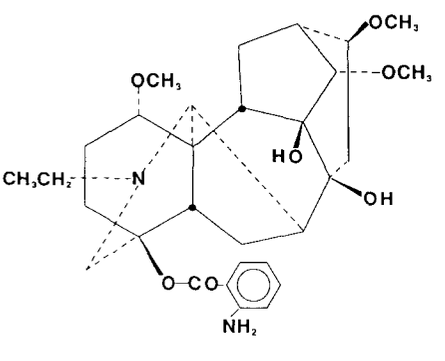
2.2. Methods

2.2.1. Experimental

The preparation of dogs and nerve recordings were performed as described in our previous studies (Rebagay and Caldwell, 1990, 1992). Briefly, adult dogs were anesthetized with morphine sulfate (2 mg/kg s.c.) and sodium pentobarbital (15–20 mg/kg i.v.). The cardiac vagal afferent nerve fibers were carefully isolated from the right cardiac nerve. Electrical activity of these fibers rose with mechanical stimulation of the left ventricular wall; therefore, their activation indicates the level of cardiac mechano-reflex receptor activation. The cardiac sympathetic efferent nerve fibers were isolated near the stellate ganglion. Both nerve activates were first amplified by an AC differential pre-amplifier before being visualized and recorded on a 2-channel digital storage oscilloscope (Nicolet, model 4094). Left ventricular pressure, heart rate, blood pressure and lead II EKG were also measured continuously. The LVP was differentiated against time to obtain LV dP/dt_{max}, an estimate of cardiac contractility. Both lappaconitine and *N*-deacetyllappaconitine were given at a dose of 150 μg/kg (i.v.) over a 30 min period. Lappaconitine was also given at a dose of 300 μg/kg in four other dogs. Lycoctonine and aconine were given at doses up to 300 μg/kg (i.v.) in two dogs. Variables were recorded from 20 min before to 60 min after beginning drug infusions.



Lappaconitine



N-deacetyllappaconitine

Fig. 1. Chemical structures of lappaconitine and *N*-deacetyllappaconitine.

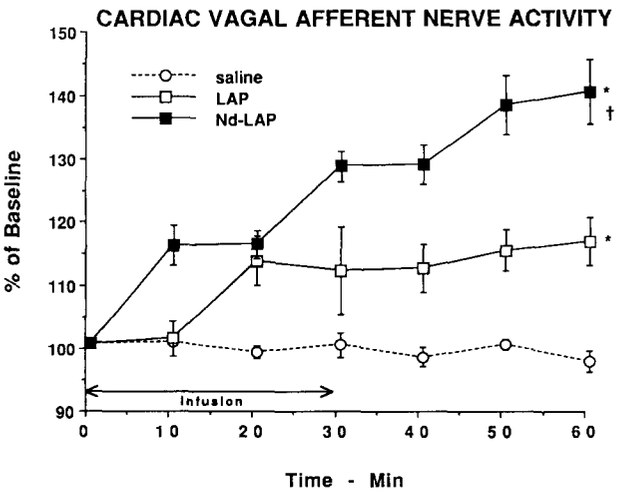


Fig. 2. Effect of lappaconitine (LAP; 150 μg/kg i.v.), *N*-deacetyllappaconitine (Nd-LAP; 150 μg/kg i.v.) and saline in cardiac vagal afferent nerve activity of dog. *Significantly different from saline treatment group ($P \leq 0.05$). †Significantly different from lappaconitine group ($P \leq 0.05$).

2.2.2. Evaluation

The data were expressed as mean ± S.E.M. Statistical evaluation for the difference among the experimental groups was performed with the Student-Newman-Keuls test and Scheffe's test after one-way analysis of variance. The differences are considered significant when $P \leq 0.05$.

3. Results

By the end of the 30 min infusion of either lappaconitine or *N*-deacetyllappaconitine at a dose of 150 μg/kg, cardiac vagal afferent nerve activity had increased significantly (Fig. 2). There was no change in

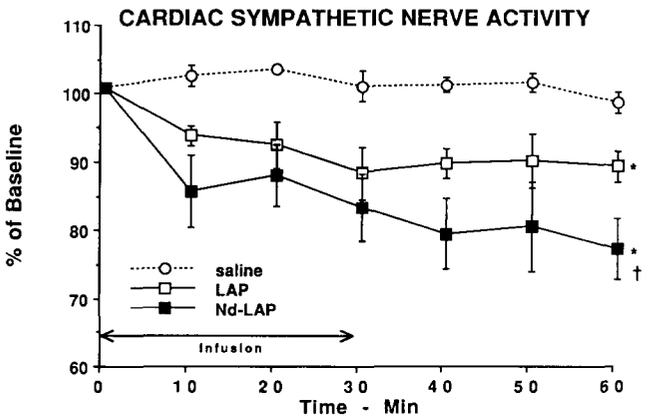


Fig. 3. Effect of lappaconitine (LAP; 150 μg/kg i.v.), *N*-deacetyllappaconitine (Nd-LAP; 150 μg/kg i.v.) and saline in cardiac sympathetic efferent nerve activity of dog. *Significantly different from saline treatment group ($P \leq 0.05$). †Significantly different from lappaconitine group ($P \leq 0.05$).

cardiac vagal afferent nerve activity in the saline-treated group. The nerve activities in the groups were significantly different from the saline group. The maximum increase of nerve activity to infusion of *N*-deacetyllappaconitine occurred at +60 min (140% of baseline) and was higher ($P \leq 0.05$) than that obtained following lappaconitine infusion (116.2% of baseline).

Both lappaconitine and *N*-deacetyllappaconitine also significantly decreased cardiac sympathetic nerve activity; saline administration did not affect cardiac sympathetic nerve activity (Fig. 3). The maximum decrease of this nerve activity (76.5% of baseline) with *N*-deacetyllappaconitine infusion was significantly more than that of lappaconitine infusion (87.5% of baseline).

Ten minutes after beginning infusion of *N*-deacetyllappaconitine, there was a significant increase in cardiac vagal afferent nerve activity and decrease in cardiac sympathetic nerve activity. However, lappaconitine did not change cardiac vagal afferent nerve activity until after 10 min of infusion (Figs. 2 and 3).

Both agents significantly decreased heart rate (Fig. 4B). The lappaconitine lowered heart rate (10%) less than did *N*-deacetyllappaconitine (16%), especially after 20 min of infusion which is correlated with the changes in cardiac vagal afferent nerve activity and cardiac sympathetic nerve activity (Figs. 2 and 3). There were decreases in diastolic blood pressure and LV dP/dt_{max} with infusion of either drug (Fig. 4A and C), although there was no significant difference between these drugs.

In an additional group of dogs, lappaconitine given at a dose of 300 μg/kg produced maximum changes in cardiac vagal afferent and sympathetic efferent nerve of $117.8 \pm 6.3\%$ and $85.6 \pm 4.7\%$ of baseline, respectively. These values were not different from those for the 150 μg/kg dose of lappaconitine, but were different from the maximum change in response to *N*-deacetyllappaconitine. Similarly, heart rate, blood pressure, and cardiac contractility responses were not different from those of the two agents at the lower dose. Lycoctonine and aconine, which differ structurally, failed to alter any of these variables in doses up to 300 μg/kg (data not shown).

4. Discussion

Certain cardiovascular effects of diterpenoid alkaloids may be due to activation of autonomic reflexes (Benn and Jacyno, 1983). The ability of these agents to alter cardiac vagal afferent nerve activity and cardiac sympathetic nerve in our experiments provides evidence for such a site for their action. The cardiac vagal

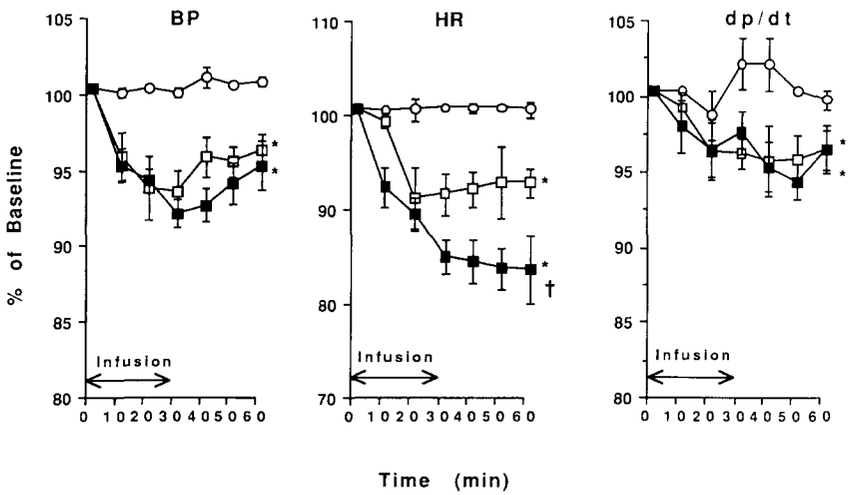


Fig. 4. Effect of lappaconitine (150 μg/kg i.v.), *N*-deacetyllappaconitine (150 μg/kg i.v.), and saline on blood pressure (BP), heart rate (HR), and left ventricular dP/dt. (○) saline; (□) lappaconitine; (■) *N*-deacetyllappaconitine. *Significantly different from saline treatment group ($P \leq 0.05$).

afferent nerve fibers in the right cardiac nerve come from reflex receptors located within the heart ventricles. Therefore, we chose cardiac vagal afferent nerve activity as an index of the actions on the cardiac reflex receptors. It is known that stimulation of cardiac reflex receptors will cause an increase in cardiac vagal afferent nerve activity and a decrease in cardiac sympathetic nerve activity (Rebagay and Caldwell, 1992). Stimulation of arterial baroreceptors also will cause a decrease in cardiac sympathetic efferent nerve activity acutely (Gillis and Quest, 1986). Therefore, changes in the cardiac sympathetic nerve activity may be due to the effects on either cardiac reflex receptors or arterial baroreceptors or both. Our data show that both lappaconitine and *N*-deacetyllappaconitine increased cardiac vagal afferent nerve activity, decreased cardiac sympathetic nerve activity, and decreased heart rate and blood pressure. These data suggest that the actions of lappaconitine and *N*-deacetyllappaconitine may be due to the stimulation of reflex receptors, at least cardiac reflex receptors.

Previous studies have indicated that certain polar aminocardenolides, such as ASI-222, work only on cardiac (ventricular) reflex receptors. On the other hand, non-polar agents, such as digoxin, act on both cardiac reflex receptors and arterial baroreceptors (Rebagay and Caldwell, 1990, 1992). The reason for the different actions between polar agent and non-polar agent may be due to the different nature of the cardiac reflex receptors and arterial baroreceptors. The cardiac reflex receptors have no lipid barrier (Miller and Kasahara, 1964), so both polar and non-polar agents may act on these structures. The arterial baroreceptors have lipid barriers (Shin et al., 1987) so only non-polar agents may interact with them. According to this hypothesis, both lappaconitine and *N*-deacetyllappaconitine should be able to act on cardiac reflex receptors. Our data have also shown that the polar agent, *N*-deacetyllappaconitine, has a much stronger effect on cardiac vagal afferent nerve activity than that of the non-polar agent, lappaconitine. This suggests that *N*-deacetyllappaconitine has more effect on cardiac reflex receptors than does lappaconitine, even when given at a larger dose. The reasons for this are not clear.

One possibility for difference in effectiveness of the agents could be differing pharmacokinetic properties. The observation that increasing the dose of lappaconitine by 2-fold did not enhance its effectiveness suggests that differences in blood level are not responsible. Another possibility relates to the interaction of various reflex receptors. It has been shown that when arterial baroreceptors input to the central nervous system is diminished, the effectness of cardiac reflex receptors is increased (Mancia et al., 1976). So possibly the greater effectiveness of *N*-deacetyllappaconitine on cardiac reflex receptors is because it cannot act on arterial baroreceptors, but only on cardiac reflex receptors. In order to understand the mechanism of the different potency between the polar and non-polar agents on the cardiac reflex receptors, further studies are required. Our data also show that the polar agent *N*-deacetyllappaconitine has more effect in decreasing cardiac sympathetic nerve activity and heart rate, whereas there are no significant differences in the effects of both agents on blood pressure and LV dP/dt_{max}. The reason for this is unknown.

We can only speculate on the inability of two other diterpenoid alkaloids to affect autonomic nerve activity and cardiovascular function. Lycotonine and aconine lack a anthranillic acid residue at position 18 and, in fact, have a carbon rather than oxygen there. However, other far more subtle differences in the structures may be responsible for the differing cardiovascular actions.

In conclusion, certain diterpenoid alkaloids activate autonomic reflex receptors, including cardiac reflex receptors. The polar agent, *N*-deacetyllappaconitine, appears to be more effective on cardiac reflex receptors than the non-polar agent, lappaconitine. Agents of this type may be of benefit in the treatment of hypertension.

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