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Central antalgic activity of resveratrol

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ABSTRACT

A single dose of resveratrol (25 μg/10 μl) was injected directly into the right lateral cerebral ventricle (icv) of Wistar rats via an implanted cannula in order to study the analgesic properties of the compound. A control group of rats received 10 μl NaCl 0.9%. The lengthening of the time to reaction to painful stimuli was assessed in the radiant heat tail-flick latency time test.

In this study, the response to painful stimuli of the animals treated with resveratrol had a bimodal profile with hypoalgesia or hyperalgesia.

In the selected experimental conditions, resveratrol had a definite analgesic effect; the increase in time to reaction ranged from 100-120% (8 rats) to 600-700% (9 rats).

In this experiment resveratrol exerts evident central antalgic effects in the majority of rats, which are related to the individual level of excitation and vigilance at baseline. Antinociceptive induced by resveratrol icv injection was maximal at 4-10 min and lasted no longer than 15 min. The effect of resveratrol to produce analgesia after a single icv injection may be interesting for preventing chronic pain.

Key words

Resveratrol • Intracerebroventricular • Analgesia

Introduction

Ever since the first epidemiological studies performed by Renaud, the evidence showed that the populations that include moderate quantities of wine in their diet are less exposed to the risk of cardiovascular events (Renaud and de Lorgeril, 1992). Subsequent studies identified resveratrol as one of the components of wine responsible for this cardioprotective activity.

Resveratrol is a phytoalexin with a chemical structure that differs from that of other polyphenols and resembles that of estradiol (Pervaiz, 2003). A number of studies carried out with resveratrol have shown that it possesses strong antiplatelet and vasodilating properties, as well as the ability to protect renal function (Bertelli et al., 2002; Chander et al., 2005; Delmas et al., 2005; Olas and Wachowicz, 2005).

Recently, resveratol has been shown to be a potent activator of sirtuins, which act in the regulation of apoptosis and cells differentiation (Howitz et al., 2003) and therefore it seems to protect neurons (Parker et al., 2005).

Of particular interest are the numerous studies that highlight the anti-inflammatory activity of this compound, which is due to prostaglandin regulation by inhibition of both cyclooxygenases (COX-1 and COX-2) (Bertelli et al., 2008; Szewczuk et al., 2004; Torres-Lopez et al., 2002). The anti-inflammatory activity of resveratrol has been well documented and can be ascribed to the inhibition of pro-inflammatory substances, such as prostaglandins. On the con-

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trary, its analgesic activity has been less studied and recent literature (Pham-Marcou et al., 2008) about this property of resveratrol, apart its involvement in COX-1 and COX-2 imbalance, do not discuss more widely the mechanisms responsible for it, which may involve other mediators besides prostaglandins, such as substance P and various cytokines.

Studies on descending inhibitory control in response to transient noxious stimulation demonstrated that there are parallel descending facilitatory mechanisms. In the rostral ventromedial medulla, two types of neurons, named "on-cells" and "off-cells", have been identified as pain modulatory neurons (Fields et al., 1991). The rostral ventromedial medulla neurons may exert bi-directionnal control of the nociception through descending serotoninergic pathways (Holden et al., 1999; Zhuo and Gebhart, 1991).

These physiological central mechanisms explain that pain is a complex phenomenon that involves both the central and peripheral nervous systems, so we decided to study the central effects of resveratrol, in order to assess whether the possible central effect on pain of resveratrol may be put in the profile already showed by other substances or mediators. For this reason we injected resveratrol directly into the central nervous system to study its central analgesic properties by recording the time to reaction (tail flick) to a standard radiant thermal stimulus.

Materials and methods

We used 40 male Wistar rats (Charles River, Calco, Italy) weighing approximately 350 g. All of the animal care and treatment procedures respected the guidelines established by Italian Government Decree No. 94/2000-A that agrees with European Community Council Directive of 24 November 1986 (86/609/EEC), and every effort was made to minimize the number of animals used and their suffering.

The animals were anesthetized with Nembutal 35 mg/kg i.p. Five minutes before anesthesia the rats received atropine 0.3 mg/kg i.p.

As previously described (Dib et al., 1998), a chronic guide-cannula of stainless steel tubing, inner diameter 0.5 mm, was implanted stereotaxically in the right lateral ventricle. The stereotaxic co-ordinates were: L = 2 mm, V = 3.5 mm and A = 6-6.5 mm. After a recovery period of at least 10 days, the correct position of the cannula was checked by an infusion of 5 µl of saline containing 1 ng of human angiotensin II (Sigma-Aldrich Chemie, St. Quentin Fallavier, France). When this octapeptide is administered into the cerebral ventricles, it induces an immediate drinking response.

Three rats were discarded because the cannula was not implanted properly (negative response to angiotensin). Ten rats with a correctly implanted cannula received 10 µl of NaCl 0.9% as sole treatment (control group). Resveratrol (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) was thoroughly dissolved in NaCl 0.9% by mechanical agitation for 4 hours.

The optimal concentration to inject into the cerebral ventricles was established to be 25 µg/10 µl in a series of preliminary tests.

The basic response to pain (mean radiant heat tail-flick latency time) was determined in each animal starting from the tenth minute before treatment with resveratrol: each animal suffered a painful stimulus every 2 minutes up to a total of 5 responses, which were averaged.

Two animals were removed from the experimental protocol, because their responses indicated a status of high analgesia already at baseline.

Twenty-five rats therefore received a single standard dose of resveratrol in their cerebral ventricles (25 µg/10 µl) and were submitted to the tail-flick test as previously described (D'Amour and Smith, 1941).

As it is known that response to painful peripheral stimuli is subjective in experimental animals when the response is modulated by central antalgic mechanisms, only the rats in which treatment with intracerebral resveratrol produced at least doubling of the maximal tail-flick time as compared to the average of the 5 pretreatment responses (mean values at baseline) were taken into consideration for the statistical analysis.

Painful stimulation was repeated every 2 minutes after intracerebral injection.

The experiment was terminated after 40 minutes *i.e.* after 21 consecutive stimulations.

Statistical analysis

Statistical analysis was performed by Anova RM, Tukey's test. Significance was assumed when $p < 0.01$.

Results

Six rats were not sensitive to resveratrol. Their time to reaction to the painful stimulus did not change substantially (mean baseline value of 2.47 ± 0.20 sec *vs*. a mean post-treatment of 1.63 ± 0.40 sec) (Fig. 1).
Two rats actually became more sensitive with a mean baseline value of 2.89 sec *vs*. a mean post-treatment of 1.27 sec (0.79 sec when only the first ten stimulations are considered).
In 17 rats the treatment with intracerebral resveratrol increased significantly the time to response to the standard painful stimulus adopted in this protocol.
As the analgesic effect usually tended to disappear within 10-15 minutes after the injection of resveratrol and its onset varied considerably from animal to another, we decided to use the longest latency time recorded in each animal (Emax) for the statistical analysis. Nine rats had a tail-flick latency time of up to 20 sec, *i.e.* 6-7 times longer than the average baseline value (17.2 ± 3.49 sec *vs*. 2.86 ± 0.52 sec; $p < 0.01$) (Fig. 2).
In other eight rats the recorded value was more than double than the value recorded at baseline (4.35 ± 0.52 sec *vs*. 1.98 ± 0.23 sec; $p < 0.01$) (Fig. 3).
At the end of the experiment the tail-flick latency time returned down to baseline in all the rats, except one of the two that had become hypersensitive.
A certain degree of hyperalgesia as compared to pretreatment values was observed in the 10 rats in which NaCl 0.9% was injected intracerebrally (controls): mean baseline pretreatment time was 2.65 ± 0.29 sec, whereas the mean latency time recorded until 28 min after the injection of NaCl 0.9% (15 consecutive stimulations) was 1.68 ± 0.12 sec. (Fig. 4).

Discussion

The subjectivity of the responses observed in this study is consistent with the bimodal responses recorded by various authors with antalgic and even algogenic substances administered directly into the central nervous system (intrathecally or intracerebroventricularly) (Dolan and Nolan, 2001; Oka et al., 1997a; Oka et al., 1997b).
It has been established that certain substances can produce both hypo-and hyper-algesia according to the quantity administered (Frederickson et al., 1978).

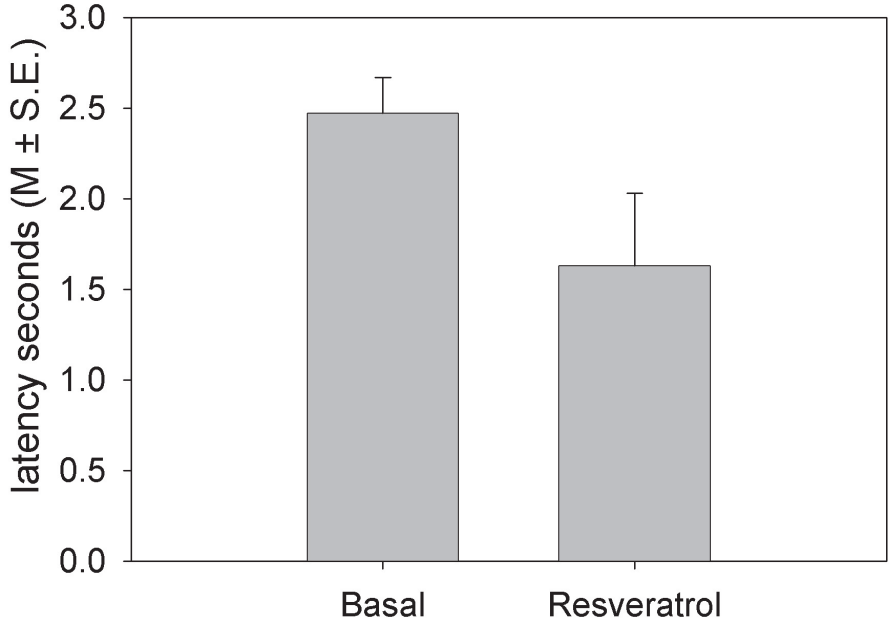

Fig. 1. - Lack of effect of resveratrol 25 μg/10 μl intracerebroventricularly injected to rats (n = 6). Basal: mean tail-flick latency time before injection. Resveratrol: mean of the latency times after treatment. p < N.S.

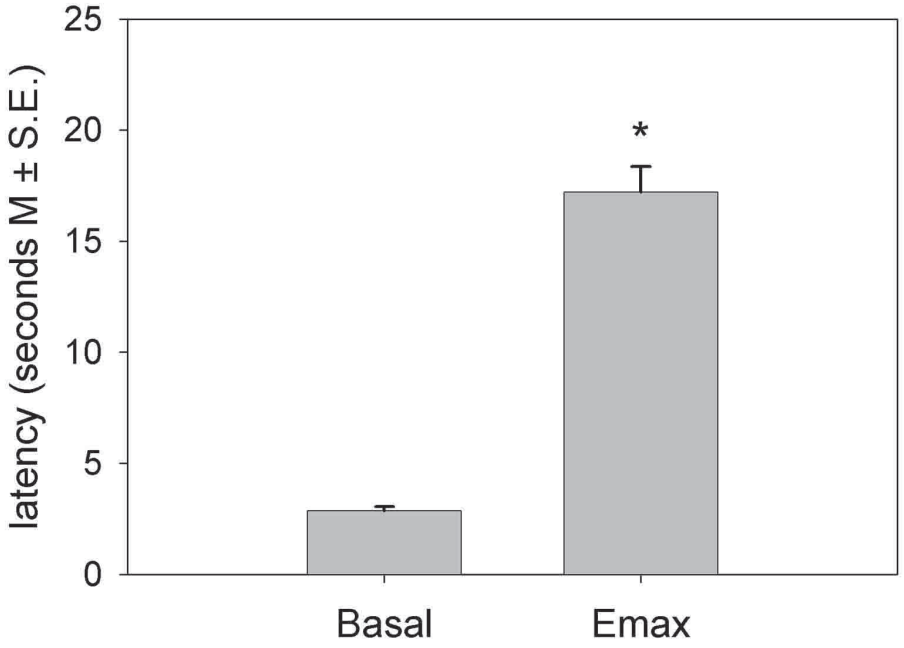

Fig. 2. - Antalgic effect of resveratrol 25 μg/10 μl intracerebroventricularly injected to rats (n = 9). Basal: mean tail-flick latency time before injection. Emax: mean of the highest latency times after injection. $p < 0.01$ Basal *vs.* Emax.

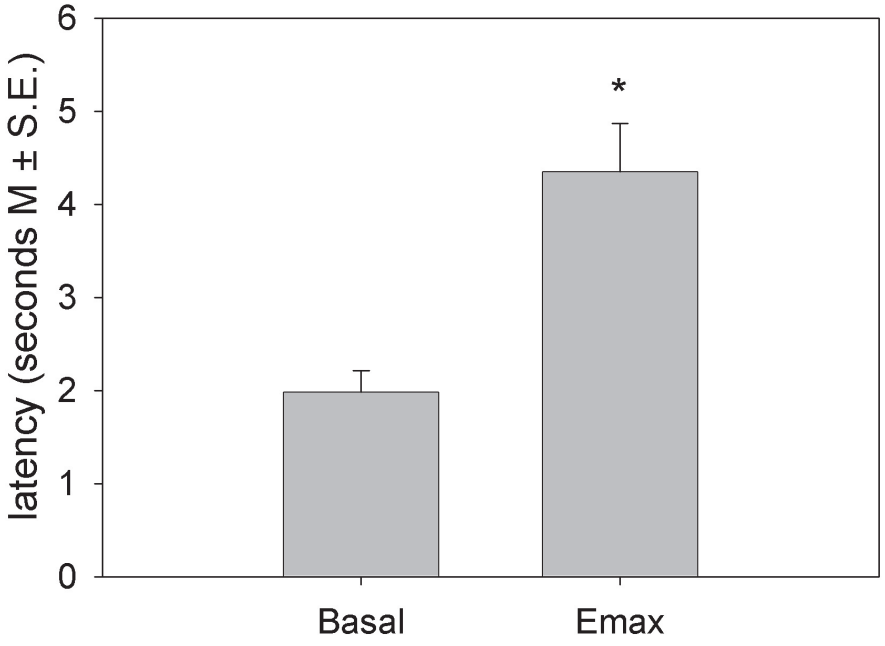

Fig. 3. - Antalgic effect of resveratrol 25 μg/10 μl intracerebroventricularly injected to rats (n = 8). Basal: mean tail-flick latency time before injection. Emax: mean of the highest latency times after injection. $p < 0.01$ Basal *vs.* Emax.

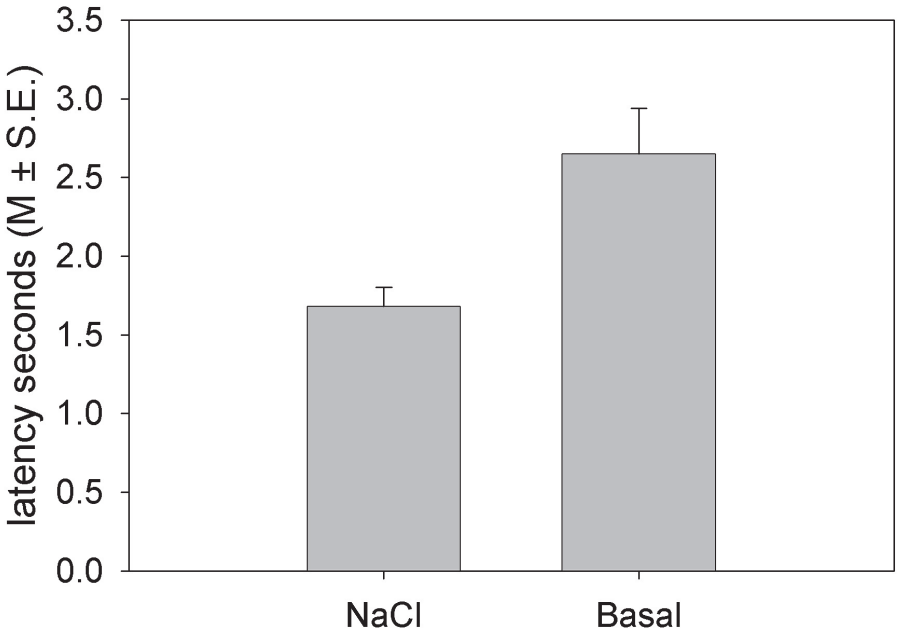

Fig. 4. - 10 µl NaCl 0.9% treated rats (n = 10) (controls). Basal: mean tail-flick latency time before injection. NaCl: mean of the latency times after treatment. p < N.S.

In this study, based on a single injection of a fixed dose of resveratrol (25 µg/10 µl), the concentration was sufficient to diminish the sensitivity to pain in 17 animals; the phenomenon was very pronounced in nine rats and less so in another eight, whereas in another six rats the concentration was probably not sufficient to modify the ability to react to painful stimuli. Finally, in two rats the dose of 25 µg of resveratrol was able to alter the mechanisms of reaction to pain in the direction of hypersensitivity.

In view of the bimodal response of rats to treatment with resveratrol (hypoalgesia in 17 rats, indifference or hyperalgesia in 8 rats), the analgesic response to resveratrol may be mediated by enkephalin activation, whereas its algogenic effect may rest on the activation of the classical mediators of pain (substance P, histamine, etc.).

The activation of the enkephalin or substance P released from the spinal cord can be facilitate by the activation of rostral ventromedial medulla serotoninergic or locus coeruleus noradrenergic descending pathways (Ren et al., 2000). The source of the inhibition can be traced back to the brain stem structures.

The biphasic (bimodal) response obtained with resveratrol isn't the first case in neuropharmacology. However, with reference to nociception, it has been demonstrated that intracerebroventricular administration of large doses of human recombinant interleukin 1 (Il-1) in rats produced short lasting analgesia as assessed by thermal nociception (hot-plate test) (Bianchi et al., 1998).

Opposite results have been obtained using lower doses of Il-1. In this perspective was found that intracerebroventricular administration of human recombinant Il-1 was able to induce hyperalgesia (Oka et al., 1993).

Similar results were previously observed after intrathecal or intracerebroventricular injected substance P or morphine that produced hypoalgesia or hyperalgesia as assessed by thermal test, the biphasic effects on thermal nociceptive thresholds depending also in this case on the dose (Jacquet and Lajtha, 1973; Frederickson et al., 1978; Dib, 1983). Also peripheral injection of 5-HT agonists showed this bimodal profile but depending upon the test used (Fasmer et al., 1986).

By operating with selective synthetic PGE2 agonists injected into the lateral cerebroventricle of

rats, it was found that brain-derived PGE2 induces mechanical hyperalgesia and hypoalgesia through the activation of the receptors EP3 and EP1, respectively (Oka et al., 1997a).

The antalgic activity of resveratrol at peripheral level is now well established (Gentilli et al., 2001; Torres-Lopez et al., 2002).

At central level, resveratrol is known to inhibit Na^+ currents in the dorsal root ganglion neurons by eliciting a hyperpolarizing shift of the steady-state inactivation voltage and lowering the recovery from inactivation of the Na^+ currents evoked at different voltages *i.e.* tetrodotoxin-sensitive and tetrodotoxin-resistant currents. It may be hypothesized that resveratrol exerts suppression or antagonism on the kinetics of Na^+ in terms of excitability of the neurons of dorsal root (Kim et al., 2005).

Moreover, recent studies confirm that the antinociceptive activity of resveratrol involves the opioidergic mechanisms. Resveratrol i.p. administered produces dose-dependent analgesia. Since pretreatment with naloxone blocks any antalgic activity and co-administration of morphine synergizes the analgesia and both morphine and resveratrol can produce tolerance, all these findings suggest that resveratrol analgesia is mediated via opioidergic pathways and that the opioid receptors involved are likely μ receptors (Helmstetter et al 1995; Gupta et al., 2004).

The resveratrol analgesic activity seems to be related to various pathways of the pain control. Cyclooxygenase inhibition and K^+ channel opening have been suggested as lying mechanisms for resveratrol-induced analgesia. On the other hand, the existence of significant tonic activity in the c-AMP-PKA signaling pathways may suggest a relationship with the biphasic effects profile of resveratrol.

In models of carrageenan evoked hyperalgesia, resveratrol intraperitoneal administration showed to induce a potent antinociceptive action on rats after 2 mg/kg (Gentilli et al., 2001) and a prolonged antinociceptive effect that lasted for at least 48 h after the treatment with doses ranging from 2 and 10 mg /kg also in the absence of any inflammatory insult evoked by the carrageenan treatment (Pham-Marcou et al., 2008).

The experimental evidences obtained by different authors by injecting resveratrol peripherally or centrally as we made showed that resveratrol acts as modulator on nociceptive transmission pathways.

From our results, we can hypothesized that the analgesia obtained after resveratrol central injection may be produce an inhibition of the cycloxygenase 2 which has been demonstrated to contribute to the anti-inflammatory properties of resveratrol (Subbaramaiah et al., 1998).

The biphasic response after resveratrol icv injection *i.e.* analgesia or hyperalgesia, as well the precise brain region involved in the analgesic or hyperalgesic effects of resveratrol remains to be explained (Frederickson et al., 1978).

However, it seems reasonable to affirm that icv resveratrol exerts analgesic effects, and resveratrol appears to be interesting for chronic pain therapy.

Further experiments will be necessary to elucidate the precise role of resveratrol on the general nociception mechanisms and its possible other effects, by taking into account that it is also well known that emotions (excitement or depression) may influence the perception of pain, considerably in man (Bar et al., 2005).

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