ANTINOCICEPTIVE PROPERTIES OF DIETHYLAMINE IN RODENTS: ROLE OF THE CANNABINOID AND OPIOID RECEPTORS

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Abstract

Diethylamine is a simple substance for which analgesic properties were proven but the underlying mechanism of action is unknown. The working hypothesis of this research was that the diethylamine could produce analgesia by interacting with the endocannabinoid system. The aim of this study was to investigate the interaction of diethylamine with cannabinoid or opioid receptors on chemical and thermal pain models in mice or rats.

Mice or rats were intraperitoneally injected with diethylamine (30-120 mg kg\(^{-1}\) bw), naloxone (10-30 mg kg\(^{-1}\) bw), AM281 (3 mg kg\(^{-1}\) bw), rimonabant (5 mg kg\(^{-1}\) bw) or saline solution as control. Analgesic effects were measured using writhing or tail-flick tests. Spontaneous locomotor activity, rectal temperature, cataleptic effects and anti-inflammatory properties after diethylamine administration were also evaluated.

Diethylamine presented analgesic properties in all used pain models. Cannabinoid receptors antagonists failed to reverse these anti-nociceptive effects. The opioid receptor antagonist, naloxone completely prevented diethylamine-induced analgesia in the tail-flick test and only partially in the writhing test. Diethylamine determined also sedation, hypothermia but no catalepsy and presented anti-inflammatory properties.

Diethylamine-induced analgesia may be related to opioid receptors stimulation but also to the anti-inflammatory properties of this simple compound which is already used as an excipient in some analgesic topical pharmaceutical products. Furthermore, the anti-nociceptive properties of diethylamine should be considered when synthesizing new analgesic molecules.

Rezumat

Dietilamina este o substanță simplă pentru care s-au descris proprietăți analgezice dar fără a se cunoaște exact mecanismul de acțiune. Una din ipotezele de lucru de la care a plecat această cercetare este că dietilamina are efect analgezic datorită unei interacțiuni cu sistemul endocanabinoizilor. Scopul acestui studiu a fost investigarea interacțiunii dintre dietilamină și receptorii canabinoizi sau opioizi cu ajutorul modelor chimice și termice de inducere a durerii la șoareci sau șobolani.

S-a injectat intraperitoneal la șoareci sau șobolani dietilamină (30-120 mg kg\(^{-1}\) corp), naloxonă (10-30 mg kg\(^{-1}\) corp), AM281 (3 mg kg\(^{-1}\) corp), rimonabant (5 mg kg\(^{-1}\) corp) sau ser fiziologic pentru loturile control. Efectul analgezic a fost evaluat cu ajutorul testului torsiunilor și testului tail-flick. S-au evaluat, deasemenea, și activitatea locomotorie...
spontană, evoluția temperaturii intrarectale precum și efectele antiinflamatorii și catalipetic la șoarece după injectarea întraperitoaneală de dietilamină.

Dietilamina a prezentat efect analgezic în toate modelele de durere utilizate. Antagoniștii receptorilor cannabinoidi nu au influențat efectul analgezic al dietilaminei. Naloxona a antagonizat complet efectul analgezic al dietilaminei în testul tail-flick și doar parțial în testul torsionilor. Dietilamina a determinat de asemenea sedare, hipotermie și a prezentat proprietăți antiinflamatorii, dar fără a avea efect catalipetic.

Analgezia determinată de dietilamina poate fi datorată stimulării receptorilor cannabinoidi dar și proprietăților antiinflamatorii demonstate pentru această molculă simplă care este deja utilizată ca excipient în diferite preparate analgezice topice. De asemenea, proprietățile analgezice ale dietilaminei ar trebui luate în considerare atunci când se sintetizează noi molecule analgezice.

**Keywords:** diethylamine, analgesia, hypothermia, naloxone, rimonabant.

**Introduction**

In the last years the endocannabinoid system has been extendedly studied for identifying new ways of obtaining cannabinoid effects as analgesia but without the psychotropic characteristic actions. The history of endocannabinoids starts in 1992 when the first endocannabinoid, the anandamide was identified [5]. In the following years other endocannabinoid molecules have been identified as 2-arachidonoyl-glycerol (2-AG) and virodhamine. Numerous roles have been described for endocannabinoids, as central and also peripheral effects: analgesia [7], increasing appetite [22], anti-inflammatory effect and vasodilatation [21].

Among the proposed methods of increasing the level of anandamide, the administration of endocannabinoid precursors or inhibitors of its metabolization were studied. The current researches are focusing on inhibition of fatty acid amide hydrolase (FAAH), the enzyme responsible of anandamide being hydrolyzed to arachidonic acid and ethanolamine [4]. URB597 (cyclohexyl carbamic acid 3′-carbamoyle-biphenyl-3-yl ester) proved to be a potent FAAH inhibitor with increasing the brain anandamide levels and amplifying the effects of this endogenous cannabinoid agonist [6]. PF3845 is one of the promising FAAH inhibitors which is now under development and it raises brain anandamide levels for up to 24 hours, producing a significant cannabinoid receptor-dependent reductions in inflammatory pain [1].

The use of possible endocannabinoid precursors was studied in a lesser extent. Knowing that endocannabinoids are derivatives of arachidonic acid conjugated with ethanolamine or glycerol it would be possible to increase the endocannabinoids levels by administering simple substances which could interact with arachidonic acid. Previous researches conducted
for evaluating the role of ethyl group in analgesia [8] discovered that the
diethylamine as an important analgesic effect at very low toxic levels. In
2008, the analgesic properties of diethylamine were patented [8] but without
knowing the mechanism of action. The diethylamine is already used as an
excipient in some topical pharmaceutical products as diethylamine-
salicylate or diethyamine-diclofenac because it facilitates the absorption of
the active drug enhancing the anti-inflammatory and analgesic effects of the
main molecules [15].

Based on the considerations above, one proposed mechanism of
action was that diethylamine could interact with arachidonic acid increasing
the brain level of anandamide due to the partial chemical structure
resemblance.

In order to test this hypothesis two important steps have been
performed. First, the cannabinoid tetrad [16] was used in mice in order to
assess the cannabinoid profile of diethylamine. The cannabinoid tetrad
consists of antinociception, sedation (spontaneous activity), hypothermia
and catalepsy. Previous researches [9] have shown that diethylamine has a
long duration and dose-dependent analgesic effect in writhing and hot-plate
tests. In the second step, the interactions between diethylamine and
cannabinoid or opioid antagonists was assessed.

**Materials and Methods**

**Animals**

Male adult Swiss albino mice (25-30 g) divided in groups of 8 or 10
and male Wistar rats (180-200 g) divided in groups of 8 were used. All the
animals were acquired from Lab Animals Hatchery of the Bucharest “Carol
Davila” University. The animals were maintained in standard laboratory
conditions with respect to Romanian regulations concerning laboratory
animals housing. Before each experiment the animals were kept in the
laboratory for at least 24 hours maintaining a light/dark cycle of 12h/12h.

All the experiment protocols were approved by the Institutional
Ethics Committee of “Carol Davila” University. The parallel design was
used for all the experiments.

**Drugs**

Substances used for the experiments were: diethylamine (Sigma-
Aldrich), AM281 (Sigma-Aldrich) and rimonabant (Cayman Chemical) –
specific CB1 receptors antagonists, naloxone (Sigma-Aldrich), an opioid
antagonist, haloperidol (Gedeon Richter), ibuprofen (Sigma-Aldrich) and
carrageenan (Sigma-Aldrich). 10% or 20% dimethylsulfoxide (DMSO)
(Sigma-Aldrich) solutions were used as a solvent for rimonabant and
AM281, respectively. All the drugs were dissolved in saline solution (NaCl 0.9%). In all experiments, saline solution (Zentiva S.A.) was used as control. The substances were administered by intraperitoneal injection.

**Analgesia assessments**

Nociception was assessed on rats and mice. On rats nociception was evaluated with a tail-flick apparatus (37360, Ugo Basile). An infrared heat stimulus was positioned below the tail on a point of 3 cm rostral to the tip of tail and the time for withdrawing the tail was recorded. The time was automatically measured by a photocell activated by the tail deflection. The cut-off time was set at 30 seconds in order to prevent tissue damage. An increase in latency time was interpreted as analgesia. In mice, the writhing test was applied. A 0.75% acetic acid solution was administered intraperitoneally and the number of writhes was counted. A significant decrease of writhes was a measure of analgesia. The analgesia assessments were done in order to evaluate the influence of naloxone (20 mg/kg bw) and AM281 (3 mg/kg bw) or rimonabant (5 mg/kg bw) on the analgesic effect of diethylamine. Naloxone was administered 90 minutes after diethylamine administration, the assessments being performed at 30 minutes after naloxone. AM281 and rimonabant were administered at 60 minutes after diethylamine injection and the analgesic effect was measured at 60 minutes after cannabinoid antagonists administration.

**Spontaneous locomotor activity**

In order to evaluate the spontaneous locomotor activity, it was used an activity cage (41 cm x 41 cm x 33 cm, 374331, Ugo Basile) which automatically recorded the number of crossings and number of rearings. The spontaneous locomotor activity was counted for 5 minutes for each animal. Between assessments the box was wiped with a 10% alcoholic solution in order to remove any particles of the previous animal and which could influence the exploration behavior of the tested mouse. A decrease of the spontaneous locomotor activity was counted as an index of sedation. Diethylamine (30, 60 and 90 mg/kg bw) effects on spontaneous locomotor activity were measured at 2 hours after administration. The influence of naloxone (20 or 30 mg/kg bw) and rimonabant (5 mg/kg bw) on the diethylamine-induced sedation was assessed at 30 minutes after naloxone administration and 60 minutes after rimonabant injection.

**Rectal temperature**

Body temperature was measured in mice with a rectal probe RET-3 (ADInstruments) inserted to a constant depth a 15 mm. The probe consists of a thin stainless steel shaft 19mm long, with a smooth ball tip of 1.7 mm diameter connected to a thermocouple thermometer (Kent Scientific Corp).


Initially, the assessments were performed immediately before (baseline) and at 1, 2, 4 or 6 hours after the intraperitoneal administration of diethylamine (30 and 60 mg/kg bw) and saline solution. Further, diethylamine (60 mg/kg bw) interactions with naloxone (10 or 20 mg/kg bw) or AM281 (3 mg/kg bw) with regard to body temperature were assessed at 30 minutes after naloxone administration and 60 minutes after AM281 administration.

**Catalepsy**

The immobility time was measured by bar’ test. The fore paws of the animals were placed on a horizontal bar positioned at 4 cm above the bench surface, and the time spent for the animals to show the cataleptic posture, which was defined as an immobile posture while keeping both forelimbs on the bar, was measured with a cut-off value of 60 seconds. Diethylamine (60 mg/kg bw), haloperidol (1 mg/kg bw) as positive control and saline solution were intraperitoneally administered 60 minutes before the assessment of the immobility time.

**Antiinflammatory assays**

**Carrageenan-induced oedema.** The antiinflammatory activity of diethylamine was evaluated by measuring the oedema induced by intraplantar (i.pl.) injection of 0.1 mL carageenan (1% w/v in saline) into the left hind paw of the rat. The right hind paw was used as control. Diethylamine (60 mg/kg bw), ibuprofen (60 mg/kg bw) as positive control and saline solution were intraperitoneally administered 15 minutes before carageenan. The paw volume was measured with a plethysmometer 7140 (Ugo Basile) immediately before and 3 and 6 hours after the intraplantar carageenan injection. For all animals both the left (injected) and right (non-injected) hind paws were measured. The increase of paw volume as compared to the baseline value was interpreted as oedema.

**Statistical analysis**

The results were expressed as means ± SEM (standard error mean). The data were statistically processed using one way analysis of variance (ANOVA), followed by the post-hoc Tuckey’s test for multiple comparisons and Dunnett’s test for comparisons with the control groups. Values lower than 0.05 of the p-coefficient ensured the statistical significance.

**Results and Discussion**

Panels A-B of figure 1 show that diethylamine (45 mg/kg bw) determines analgesia in writhing test with a decrease of 90% of the writhes numbers as compared with control. In tail-flick test, diethylamine (100 mg/kg bw) doubled the latency of tail deflection (see figure 2 panels A-B). As shown in Figure 1A and 2A, the CB1 receptor antagonists, AM 281 (3
mg kg\(^{-1}\) bw) and rimonabant (5 mg/kg bw) had no effect per se and did not prevent antinociception induced by diethylamine. The opioid antagonist, naloxone (20 mg/kg bw) reversed the diethylamine-induced antinociception in tail-flick test and reduced the analgesia determined by diethylamine from 90% to 60% as compared to control (figure 1B and 2B).

**Figure 1**

A. Anti-nociceptive effect of diethylamine (DEA 45 mg/kg bw) and AM281 (3 mg/kg bw) on writhing test. B. Anti-nociceptive effect of diethylamine (DEA 45 mg/kg bw) and naloxone (Nalox 20 mg/kg bw) in the writhing test. The columns represent the mean values of the number of writhes done by each group after substances administration and the error bars represent SEM. n=10 mice per group. * p<0.05 as compared with the diethylamine groups. **p<0.005 as compared with the control groups (saline solution or 20% DMSO), one-way ANOVA followed by Dunnet or Tuckey tests.

**Figure 2**

A. Anti-nociceptive effect of diethylamine (DEA 100 mg/kg bw) and rimonabant (Rimo 5 mg/kg bw) on the tail-flick test. B. Anti-nociceptive effect of diethylamine (DEA 100 mg/kg bw) and naloxone (Nalox 20 mg/kg bw) on the tail-flick test. The columns represent the mean latencies of tail deflection after substances administration. n=8 rats per group. **p<0.005 as compared with the control groups (saline solution or 20% DMSO), one-way ANOVA followed by Dunnet or Tuckey tests.
As shown in figure 3 – panel A, diethylamine (30, 60 and 90 mg/kg bw) significantly reduced the spontaneous locomotor activity 2 hours after administration by decreasing the number of orizontal crossings as compared to the control. The most important decrease in the spontaneous locomotor activity was determined by the higher dose of naloxone which reduced the number of horizontal crossings about 10 times as compared to the control (52.00±38.63 vs. 594.86±45.45). Naloxone and AM281 did not influence the sedative effect of diethylamine.

Regarding catalepsy, figure 3 – panel B, diethylamine did not modify the immobility time as compared to the control (2.27±1.40 seconds vs. 0.57±0.79 seconds). Haloperidol, which was used as positive control, showed a strong cataleptic effect compared with both control and diethylamine groups.

**Figure 3**

A. Sedative effect of diethylamine (DEA 30, 60, 90 mg/kg bw) evaluated by number of orizontal crossings done in an activity cage. B. Cataleptic effect of diethylamine (60 mg/kg bw) in comparison with haloperidol (1 mg/kg bw). C. The effect of diethylamine on body temperature evaluated at 1, 2, 4 and 6 hours after substances administration. D. Effect of diethylamine (60 mg/kg bw) associated with naloxone on body temperature. n=8-10 mice per group. *p<0.05 as compared with the control group which received saline solution, one-way ANOVA followed by Dunnet or Tuckey tests. **p<0.005 as compared with the control groups (saline solution or 20% DMSO), one-way ANOVA followed by Dunnet or Tuckey tests.
The low dose of diethylamine did not induce hypothermia while the higher dose of diethylamine (60 mg kg\(^{-1}\) bw) significantly decreased mice’s body temperature which lasted for at least 6 hours depicted in figure 3 – panel C. Diethylamine determined a body temperature of 35.41±0.76\(^\circ\)C at 1 hour after administration, value which remains approximately constant during each measurement. AM281 did not influence the diethylamine induced hypothermia while naloxone induced a decrease of hypothermia intensity but without statistical significance (figure 3 – panel D).

Before carrageenan injection there was no significant difference in volume between right and left hind paws of the rat. Intraplantar injection of carrageenan 1% w/v resulted in time-related increase in left hind paw volume. The right hind paw volume was used as control and it did not change significantly thought the experiment. Diethylamine (60 mg kg\(^{-1}\) bw) and ibuprofen (60 mg kg\(^{-1}\) bw) administered 15 minutes before carrageenan inhibited paw oedema at 3 hours after carrageenan injection when the oedema was reduced with 37% and 47% respectively (Figure 4). At 6 hours after carrageenan injection ibuprofen failed to inhibit the paw oedema but diethylamine preserved its anti-inflammatory effect observed at 3 hours.

![Figure 4](image.png)

The anti-inflammatory effect of diethylamine (DEA 60 mg/kg bw) at 3 and 6 hours after administration as compared with ibuprofen (60 mg/kg bw). n=8 rats per group. Each symbol represents the mean±SEM. * p<0.05 as compared to the control group. ** p<0.005 as compared to the control group, one-way ANOVA followed by Dunnet or Tuckey tests, as appropiate.

In this study, intraperitoneal administration of diethylamine induced analgesia, sedation, hypothermia and no catalepsy. The diethylamine analgesic effect was antagonized only by naloxone and not by cannabinoid
antagonists while the sedative effect was not reversed by the opioid or cannabinoid antagonists. Also only naloxone had the tendency to reduce the hypothermia determined by diethylamine. Because diethylamine showed positive results only in three tests out of four tests which form the tetrad model and these effects were not reversed by cannabinoid antagonists, the interaction between diethylamine and the cannabinoid system was ruled out. Due to the fact that naloxone reduced or reversed the analgesic effect of the diethylamine a new possible mechanism of action for the diethylamine was proposed. The results of this study demonstrate that diethylamine induces analgesia and possibly the hypothermia most probably by interacting with the opioid system. Because diethylamine induced sedation was not reversed by any tested cannabinoid and opioid antagonists, the conclusion is that diethylamine determines sedation in mice but the underlying mechanism is unknown.

The analgesic effect of diethylamine was proved in two models of pain: the writhing test which can assess the analgesic effect of non-steroidal anti-inflammatory drugs and the tail-flick test which refers predominantly to a spinal reflex. The fact that the diethylamine’ analgesic effect was reversed in both tests by naloxone indicates a possible interaction between diethylamine and the opioid system as the main mechanism of action. But the results obtained in the anti-inflammatory assay showed that diethylamine has an anti-inflammatory effect which could be partially responsible for the analgesic effect proven in the writhing test. The anti-inflammatory effect of diethylamine explains the naloxone induced partial reversion of the analgesic effect in the writhing test. Naloxone antagonized only the opioid component of the diethylamine analgesic effect and not on the anti-inflammatory component. Thus it can be conclude that the diethylamine induced analgesia is due to two mechanisms of action, the enhancing effect of diethylamine on the opioid system activity and the anti-inflammatory effect of this compound.

Diethylamine elicited also a hypothermic effect but only naloxone presented a tendency to reverse it and not at all by AM281 which lead to the possible implication of the opioid receptors in the hypothermic effect of diethylamine. It is known that the opioids as morphine and meperidine can induce an early hypothermia followed by a late hyperthermia in rodents [3] but the underlying mechanism involves not only the opioid receptors but also the serotoninergic system [13, 20]. Other studies showed that morphine produced hyperthermia at low doses and dose-related hypothermia at higher doses with this effect being influenced by drugs which alter the dopamine system [2]. Based on the presented results and the existing data, the
diethylamine induced hypothermia could be determined by interactions with
the opioid system but it could also be determined by another mechanism of
action as the interaction with the serotoninergic or dopaminergic systems.

The data regarding the biological effects of diethylamine are only a
few thus the present study is well justified and the results represent one
step ahead to establish the effects and the mechanism of action of this
simple compound. Diethylamine can cause a rapid voltage-dependent,
cardiac and skeletal sodium open-channel block and reduces the probability
of channel closure producing a shift of the steady-state activation curve
toward more hyperpolarizing potentials [23]. Because of these previous
existing data, the initial working hypothesis was that diethylamine could
determine analgesia by blocking sodium-channels. It is well-known the
efficacy of sodium channel blockers as carbamazepine, phenytoin in
neuralgias [18] and the use of lidocaine as an local anesthetich [19] but the
possibility that the diethylamine would determine analgesia through this
mechanism is counteracted by the very weak sodium channel blockage
produced by diethylamine which has been considered insufficient for
inducing such a strong analgesic effect.

Diethylamine is also a metabolism product of disulfiram [10], a
substance which can cause significant changes in several neurochemical
parameters, including levels of P-like substance and additionally, changes in
levels of monoamines and their metabolites [12, 14] or by changing the
brain level of P substance. Recent data proved that oral disulfiram presents
anti-inflammatory properties due to the inhibition of the nuclear factor
kappa B-dependent pathway and by suppression of the subsequent
production of pro-inflammatory mediators [11]. Based on the results of the
present study a possible additional explanation of the disulfiram’ anti-
inflammatory effect could be the anti-inflammatory properties of
diethylamine, one of the metabolism products of disulfiram.

Conclusions

In conclusion, the results of the present study indicate that
diethylamine has an analgesic effect mediated by two components: a central
component represented by the activation of the opioid system and a
peripheral component due to an anti-inflammatory effect. The opioid
receptors could also partially be involved in the diethylamine induced
hypothermia. Opioid or cannabinoid receptors are not involved in the
sedative effect of diethylamine and further studies are required in order to
elucidate the mechanisms responsible of this effect. Because diethylamine
was used until now only as an excipient, the further utilization of this
compound should take into account the presented results which could also be considered when synthesizing new complex analgesic molecule.

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References
6. Fegley D., Gaetani S., Duranti A., Tontini A., Mor M., Tarzia G., Piomelli D., Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3'-carbamoyl-biphenyl-3-yl ester (URB597); effects on anandamide and oleoylethanolamide deactivation. J Pharmacol Exp Ther., 2004; 313: 352-358.

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