Amino Acids (2006) 30: 233–242
DOI 10.1007/s00726-005-0270-1

Amino Acids
Printed in Austria

GABA-mediated effects of some taurine derivatives injected i.c.v. on rabbit rectal temperature and gross motor behavior

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Received November 7, 2005
Accepted November 13, 2005
Published online April 4, 2006;

Summary. Some synthetic taurine analogues, namely ethanolamine-*O*-sulphate (EOS), *N*,*N*-dimethyltaurine (DMT), *N*,*N*,*N*-trimethyltaurine (TMT) and 2-aminoethylphosphonic acid (AEP) were shown to interact with rabbit brain $GABA_A$- or $GABA_B$-receptors, while (±)piperidine-3-sulfonic acid (PSA) inhibited the activity of rabbit brain 4-aminobutyrate transaminase. This suggests that they behave like direct/indirect GABA agonists or GABA antagonists and affect thermoregulation and gross motor behaviour (GMB) which are under GABA control. In the present study micromole (1.2–48) amounts of these compounds were i.c.v. injected in conscious, restrained rabbits while monitoring rectal temperature (RT), ear skin temperature (EST) and GMB. AEP, EOS, DMT and TMT induced a dose-related hyperthermia, ear vasoconstriction and excitation of GMB, while PSA induced a dose-related hypothermia, ear vasodilation and inhibition of GMB. EOS antagonized in a dose-related fashion hypothermia induced by 60 nmol THIP, a $GABA_A$ agonist, while AEP, DMT and TMT counteracted that induced by 8 nmol R(-)Baclofen, a $GABA_B$ agonist.

In conclusion, EOS and AEP, DMT, TMT seem to act as $GABA_A$ and $GABA_B$ antagonists, respectively, while PSA behaves like an indirect GABA agonist, all affecting the central mechanisms which drive rabbit thermoregulation.

Keywords: Taurine – Taurine derivatives – GABA – $GABA_A$ – $GABA_B$ agonist/antagonist – Thermoregulation

Abbreviations: AEP, 2-aminoethylphosphonic acid; DMT, *N*,*N*-dimethyltaurine; EOS, ethanolamine-*O*-sulphate; EST, ear skin temperature; GABA, γ-aminobutyric acid; GMB, gross motor behaviour; Muscimol, 5-aminomethyl-3-hydroxyisoxazole; POAH, preoptic region of the rostral hypothalamus; PSA, (±)piperidine-3-sulfonic acid; R(-)Baclofen, (R)-4-amino-3-(4-chlorophenyl)butanoic acid; RT, Rectal Temperature; SMC, sensorimotor cortex; TAU, 2-aminoethanesulfonic acid, taurine; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; TMT, *N*,*N*,*N*-trimethyltaurine

Introduction

Amino acids have received increased attention with regard to their thermoregulatory effects and possible role as neurotransmitters within the thermoregulatory system. There is strong evidence that both taurine and GABA participates in the neuronal network that down-regulates body temperature (DeFeudis, 1984; Yakimova et al., 1996; Pierau et al., 1997; Frosini et al., 2000, 2003a, b, 2005). In particular, taurine, when injected i.c.v., induces dose-related hypothermia accompanied by depression of gross motor behavior and peripheral vasodilation (Sgaragli et al., 1981; Frosini et al., 2003b), whereas the taurine antagonist 6-aminomethyl-3-methyl-4H-1,2,4-benzothiadiazine-1,1-dioxide (TAG), increases core temperature (Sgaragli et al., 1994; Frosini et al., 2003b). Central or systemic injection of GABA, $GABA_A$ or $GABA_B$ receptors agonists, as well as that of the GABA-transaminase inhibitor vigabatrin, diminishes core temperature, whereas the injection of antagonists of either class of receptors induces hyperthermia (Serrano et al., 1985; Jackson and Nutt, 1991; Ginefri-Gayet and Gayet, 1993; Frosini et al., 2004). In the conscious rabbit, exposure to heat stress modifies brain metabolism of taurine and GABA so that higher amounts of both aminoacids were found in the cerebrospinal fluid: this was assumed as a response aimed at counteracting the resulting hyperthermia (Frosini et al., 2000, 2005). Moreover, during IL-1 β-induced fever, to reset the hypothalamic set point towards higher value(s), a down-regulation of brain GABA metabolisms is required (Frosini, 2005). Concurrently, in febrile animals, taurine can play a protective role against fever-associated events such as excitatory influences or electrolyte alterations (Frosini, 2005). I.c.v. injection of taurine in rabbits, in fact, was shown to reduce the febrile response caused by leukocytic pyrogen

or prostaglandin E_2 (Sgaragli et al., 1981). A recent study from this laboratory indicates that a specific taurinergic pathway mediates the effects of taurine on thermoregulation in the rabbit (Frosini et al., 2003b).

The pathways that down-regulate body temperature are of overwhelming interest in view of the effectiveness of hypothermia in protecting brain from ischaemia-reperfusion damage. Post-ischaemic hypothermia, in fact, is particularly effective at attenuating ischaemia-related tissue injury and behavioral deficits (Coulburne and Corbett, 1995). The observation that delayed body cooling still resulted in cerebral protection, suggests that hypothermia affects not only the injury mechanisms in the early ischaemic cascade (Olsen et al., 2003), but also the later occurring inflammation that develops after the activation of microglia and resident perivascular and parenchymal macrophages and infiltration of inflammatory cells (Si et al., 1997; Han et al., 2002). Although there is a growing body of experimental evidence, which indicates protection of brain against ischaemic damage by hypothermia, its mechanism of action is still undefined. To this end, pharmacological manipulation of endogenous cryogens might represent a useful therapeutic strategy.

In a previous study, the affinity of 19 taurine analogues towards $GABA_A$ and $GABA_B$ receptors or taurine binding site has been studied in rabbit brain synaptosomal preparations and some direct taurinergic compounds (namely 2-aminoethylarsonic acid (AEA), (±)*cis*-2-aminocyclohexane sulfonic acid (CAHS), 2-hydroxyethanesulfonic acid (ISE) and TAG) were identified (Frosini et al., 2003a). These compounds proved crucial in defining the role of taurine in the CNS. In fact, by studying their effects on body temperature in the conscious rabbit it was possible to demonstrate the existence of a specific taurinergic pathway involved in the central mechanisms of thermoregulation (Frosini et al., 2003b).

Among the derivatives studied, however, some compounds namely ethanolamine-*O*-sulphate (EOS), *N*,*N*-dimethyltaurine (DMT) and *N*,*N*,*N*-trimethyltaurine (TMT) and 2-aminoethylphosphonic acid (AEP) were found to bind to $GABA_A$- or $GABA_B$-receptors, while (±)piperidine-3-sulfonic acid (PSA) inhibited the activity of 4-aminobutyrate transaminase (i.e. GABA-transaminase, EC 2.6.1.19) (Frosini et al., 2003a), thus suggesting that they could act as GABA agonists/antagonists.

The aim of the present work was to verify whether EOS, DMT, TMT, AEP and PSA are able to modify rabbit body temperature (RT) and gross motor behavior (GMB). The results showed that AEP, EOS, DMT and TMT induced a dose-related hyperthermia, ear vasoconstriction and excitation of GMB, while PSA induced a dose-related hypothermia, ear vasodilation and inhibition of GMB. EOS antagonized in a dose-related fashion hypothermia induced by 60 nmol THIP, a $GABA_A$ agonist, while AEP, DMT and TMT counteracted that induced by 8 nmol R(-)Baclofen, a $GABA_B$ agonist. These taurine derivatives seem to act as $GABA_A$/$GABA_B$ antagonists or, in the case of PSA, as indirect GABA agonists in the central mechanism which drive rabbit thermoregulation.

Materials and methods

Materials

AEP and EOS were purchased by Sigma-Aldrich (St Louis, MO, USA), while R(-)Baclofen ($GABA_B$ agonist) and THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol, $GABA_A$ agonist) from R.B.I. (Netik, MA, USA).

DMT was prepared by methylation of taurine as described by Clark et al. (1933). TMT was prepared starting from DMT, which was methylated with iodomethane in the presence of a hindered base (tributylamine) (Barnhurst, 1961). PSA was prepared by catalytic hydrogenation (nickel as a catalyst) of sodium pyridine-3-sulfonate as reported by Freifelder and Wright (1964). The purity of compounds was evaluated above 95% by ^{1}H NMR or High Perfomance Liquid Chromatography (HPLC).

Animals

Adult male New Zealand albino rabbits (Charles River Italia, Italy) weighing 2.0–2.5 kg were kept in large individual cages under a 12 h:12 h, day-night cycle at 20°C ambient temperature (Ta). Drinking water and conventional laboratory rabbit food were available ad libitum. Before the experimental session, the animals were habituated to restraint and to the rectal probe in order to minimize the stress response.

Surgery

All the experiments were performed in strict compliance with the recommendations of the EEC (86/609/CEE) for the care and use of laboratory animals and were approved by the Animal Care and Ethics Committee of the University of Siena, Italy.

At least 3 weeks before the experiment, animals were implanted with a cannula for intracerebroventricular (i.c.v.) injection at the level of the lateral ventricle. The stereotaxic technique has been described elsewhere (Frosini et al., 2003b). Anaesthesia was induced by i.m. injection of xylazine chloride (10 mg kg^{-1}, Rompun® vet., Bayer AG, Germany) and ketamine hydrochloride (35 mg kg^{-1}, Ketavet®, Parke Davis/Warner-Lambert, USA). After surgery, rabbits were injected for at least 5 days with the following drugs: prednisolone acetate (Novosterol®, Vetem S.p.A., Italy), 10 mg/day i.m.; enrofloxacin, (Baytril®, Bayer AG, Germany) 25 mg/day i.m. The animals were allowed to recover for at least 15 days.

Experimental protocol

As soon as rabbits had recovered from the surgical operation, in order to reduce artefacts arising from animal restraint, manipulation or i.c.v. injection, they were habituated to remain quiet in the restraining cage that holds the neck of the animal while allowing the movement of the trunk and posterior legs. All experiments started between 08.30 and 09.30 h to avoid

the bias on measurements caused by diurnal rhythms. Conscious animals were individually housed in a thermostated chamber set at neutral temperature (20°C). Concurrently, ear skin- and rectal-temperature (EST and RT) were measured to an accuracy of 0.1°C, using thermocouples attached to the ear and inserted 10 cm deep into the rectum, respectively, every 5 min. The thermocouple thermometer was connected to a personal computer with an Isothermex program (Columbus Instr., Columbus, OH, USA). Temperature was monitored for at least 1 h before the experimental session and up to 325 min after the treatment. RT was again monitored 24 h after i.c.v. injection. No animal was used more often than once a week.

At the peak of RT change which followed i.c.v. drug injection, GMB was monitored. The time of peak hypothermia or hyperthermia was that wherein two subsequent readings of RT were equal or the second one was slightly higher or lower, respectivelly. Shortly thereafter, animals were taken out from the thermostatted chamber and removed from the restraining cage by an observer attending the experimental session and not aware of the treatment, positioned on a table and scored for GMB according to the following arbitrary scale (Frosini et al., 2003b; 2004): 0, no effect; −, sedation and slight motor incoordination; −−, sedation and motor incoordination; − − −, sedation and long lasting motor incoordination; − − − −, sedation, severe and long lasting motor incoordination and loss of righting reflex; +, alertness; ++, alertness and increased panting rate (50–75 breaths/min); +++, alertness and elevated panting rate (75–100 breaths/min); ++++, alertness, sustained panting rate (breath/min >100) and episodic convulsive attacks. GMB scoring procedure took place in a 5–10 min period.

Compounds dissolved in pyrogen-free water were administered i.c.v. in a final volume of 10 or 20 µl with an Agla micrometer-operated syringe (Burroughs Wellcome and Co., London, UK), to randomly selected rabbits (4–6 animals/group/dose). Among all the rabbits used in this study, six were randomly selected to form the control group in which the vehicle was injected i.c.v. and RT monitored.

Statistical analysis

Values are expressed as mean ± s.e. mean and reported as changes in RT or EST (ΔRT and ΔEST). The area under the experimental curve (AUC) relative to RT was calculated by a combined linear logarithmic trapezoidal method using Graphpad-Prism 3.0 program (GraphPad Software Inc., San Diego, CA, USA). The comparison between $AUC_{(0-24\,h)}$ of control (vehicle-injected) vs treated animals or between the $AUC_{(0-24\,h)}$ relative to the different doses of the compounds, was performed using ANOVA followed by post-hoc Dunnett's test. A P value <0.05 was considered significant.

Results

Effects of i.c.v. injection of pyrogen-free water on RT, EST and GMB

To isolate any experimental artifacts arising from animal restraint, manipulation or i.c.v. injection, in a group of 6 rabbits, kept at neutral ambient temperature (20°C) and treated by i.c.v. injection of 10 µl pyrogen-free water, RT and EST were monitored. RT and EST basal values were, respectively, 39.1 ± 0.2 and 20.8 ± 0.2°C. Pre- and post-injection values of temperatures showed no statistically significant differences (data not shown). GMB was not affected by the treatment.

Effects of i.c.v. injection of AEP, EOS, DMT, TMT and PSA on RT, EST and GMB

As reported in Fig. 1A, at the dose of 1.2×10^{-6} mol AEP had no effects on RT (n = 6) while, at the dose of 1.2×10^{-5} mol, it induced hyperthermia which peaked with a ΔRT value of 1.4 ± 0.3°C at 300 min ($AUC_{0-24\,h}$ AEP vs control, $P<0.01$, n = 6). 24 h after treatment RT had returned to basal values. An even higher hyperthermic response was induced by the 4.8×10^{-5} mol dose (n = 4), with a time-course of the increase in RT which was very close to that observed with the previous one, peaking at 300 min with a ΔRT value of 1.5 ± 0.1°C. After 24 h, however, animals of this group were still hyperthermic, RT regaining basal values only 48 h after treatment (data not shown). The increase in RT observed at this dose was accompanied by a moderate and transient decrease in EST which peaked with -ΔEST value of 2.5 ± 0.5°C at 25 min, then regaining basal values which remained stable thereafter. With all doses, AEP did not significantly affect GMB.

The effects of EOS have been assessed at the doses of 1.2×10^{-7}, 1.2×10^{-6}, 1.2×10^{-5} and 4.8×10^{-5} mol (n = 4–6 rabbits/dose). As shown in Fig. 1B, EOS did affect RT only at the highest dose, causing hyperthermia, which peaked with a ΔRT value of 0.9 ± 0.3 at 325 min ($AUC_{0-24\,h}$ EOS vs control: $P<0.05$, n = 6). Hyperthermia was accompanied by a sustained decrease in EST which peaked at 175 min with a -ΔEST value of 3.7 ± 0.9°C and remained stable around this value thereafter. After 24 h all animals had regained RT basal values. EOS did not affect GMB.

PSA was tested at the doses of 1.2×10^{-5} and 4.8×10^{-5} mol (Fig. 1C). At the lower dose this compound induced a slight hypothermia (-ΔRT max of 0.5 ± 0.1°C at 75 min, n = 4) with a return to basal values at 150 min. The relative $AUC_{0-24\,h}$, however, was not significantly different from that related to vehicle injection. On the contrary, with the 4.8×10^{-5} mol dose, PSA significantly lowered body temperature ($AUC_{0-24\,h}$. $P<0.01$ vs control, n = 4). RT, in fact, decreased regularly attaining a -ΔRT max value of 1.4 ± 0.4°C at 100 min; the recovery of baseline values was reached at 200 min post-treatment. GMB was also affected and rabbits exhibited sedation, drowsiness and motor incoordination (arbitrary scale, −−).

DMT was tested at the doses of 1.2×10^{-8}, 1.2×10^{-7}, 1.2×10^{-6}, 1.2×10^{-5} and 4.8×10^{-5} mol (Fig. 2A). At either 1.2×10^{-8} and 1.2×10^{-7} mol dose, it did not modify RT ($AUC_{0-24\,h}$ DMT vs control, NS, n = 4/dose). At

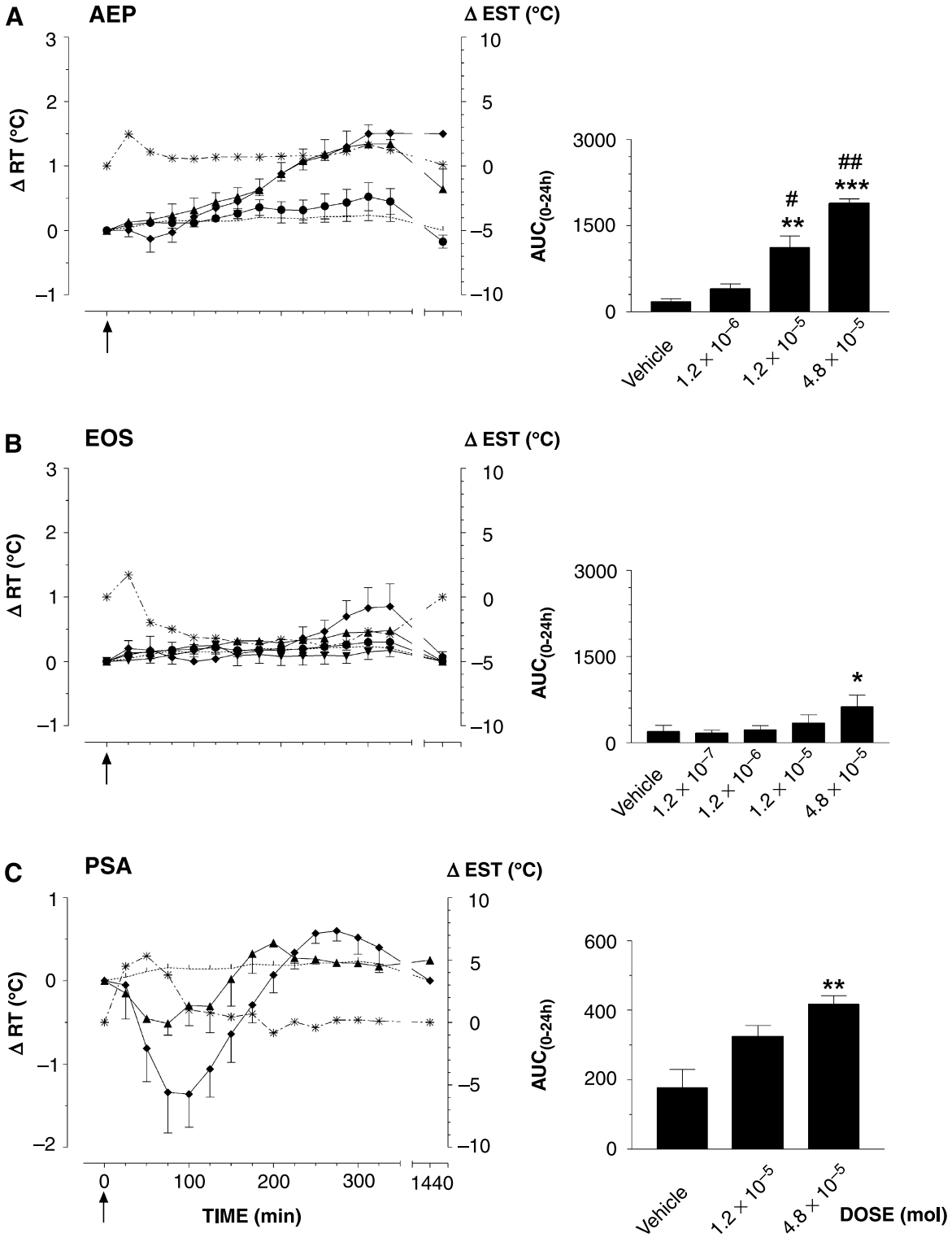

Fig. 1. Mean (± s.e. mean) changes in rabbit rectal- (RT) and ear skin temperature (EST) following i.c.v. administration of AEP (**A**), EOS (**B**) and PSA (**C**). The doses injected were 1.2×10^{-7} (▼), 1.2×10^{-6} (●), 1.2×10^{-5} (▲) and 4.8×10^{-5} mol (◆). Vehicle alone (pyrogen-free water, dotted line) was injected at the same volume used for the compounds to a group of six rabbits. EST was measured in animals treated with the highest dose (∗, dotted line). To improve clarity, s.e.mean of vehicle RT values and EST values are not depicted. Arrows indicate the time of i.c.v. injection. In the right panels the area under the experimental curve ($AUC_{(0-24\,h)}$) of RT changes relative to each dose/compound is shown. The comparison between $AUC_{(0-24\,h)}$ of vehicle-injected vs AEP-, EOS- or PSA-injected rabbits or between $AUC_{(0-24\,h)}$ of the different doses of each compound was performed by using Student's t test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs vehicle. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs the previous dose

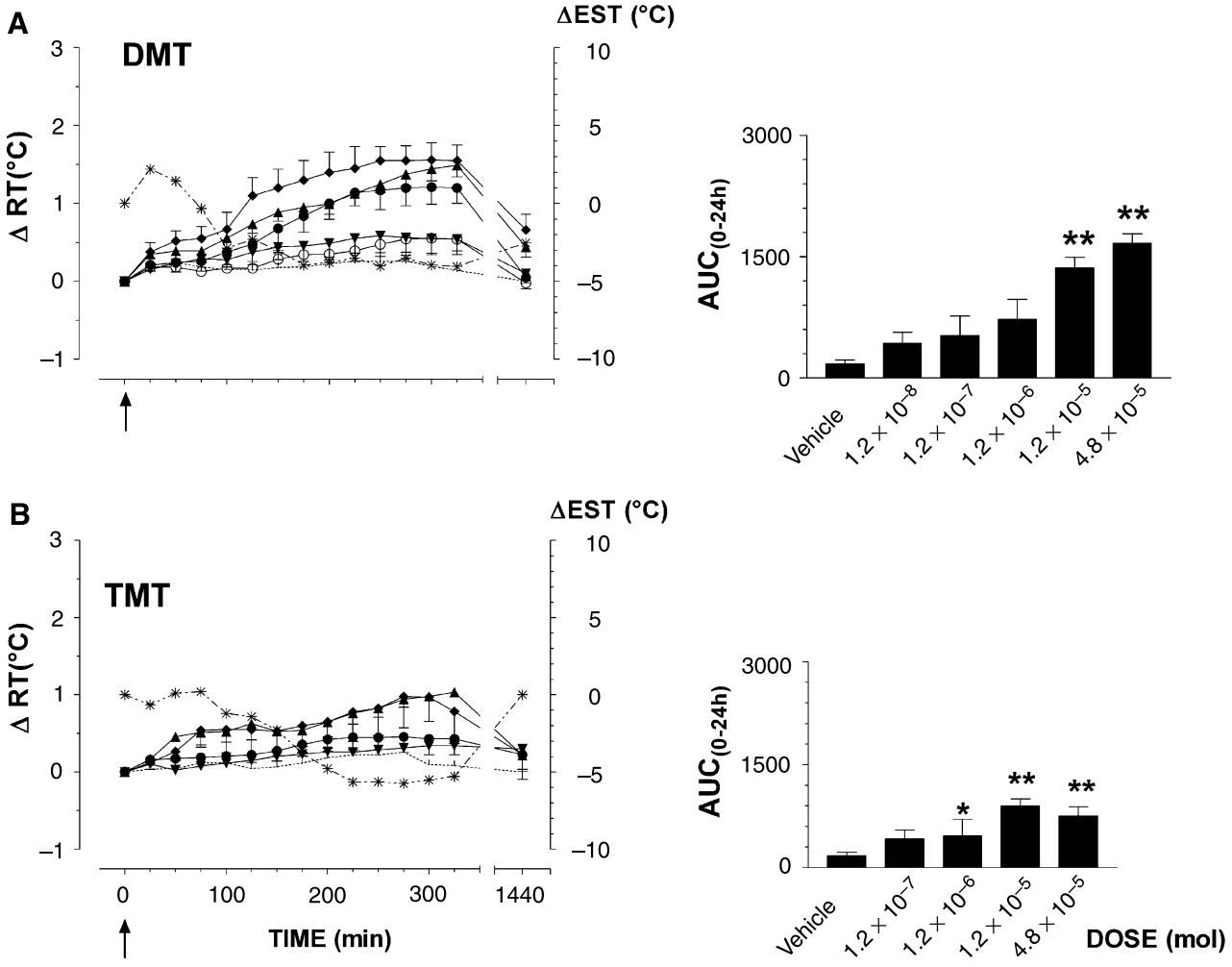

Fig. 2. Mean (± s.e. mean) changes in rabbit rectal- (RT) and ear skin temperature (EST) following i.c.v. administration of DMT (**A**) and TMT (**B**). The doses injected were 1.2×10^{-8} (○, n = 6), 1.2×10^{-7} (▼, n = 5), 1.2×10^{-6} (●, n = 4), 1.2×10^{-5} (▲, n = 3) and 4.8×10^{-5} mol (◆) mol. Vehicle alone (pyrogen-free water, dotted line) was injected at the same volume used for the compounds to a group of six rabbits. EST was measured in animals treated with the highest dose (∗, dotted line). To improve clarity, s.e.mean of vehicle RT values and EST values are not depicted. Arrows indicate the time of i.c.v. injection. In the inset the area under the experimental curve ($AUC_{(0-24h)}$) of RT changes relative to each dose is shown. The comparison between $AUC_{(0-24h)}$ of vehicle-injected vs DMT- or TMT-injected rabbits was performed by using Student's t test. *P < 0.05, **P < 0.01 vs vehicle

the 1.2×10^{-6} mol dose, however, it induced a slight hyperthermia with a ΔRT_{max} value of 1.2 ± 0.2°C at 325 min ($AUC_{0-24\,h}$ DMT vs control, $P < 0.05$, $n = 4$). The hyperthermia was more sustained at 1.2×10^{-5} and even more at 4.8×10^{-5} mol dose ($AUC_{0-24\,h}$ DMT vs control, $P < 0.01$, $n = 4$/dose). In particular, the 4.8×10^{-5} mol dose-induced hyperthermia was accompanied by a deep vasoconstriction ($-\Delta EST_{max} = 4.0 \pm 1.1$°C at 250 min), which lasted for the entire experimental session. DMT at all the doses tested, induced moderate changes of GMB consisting in alertness and increased panting rate (arbitrary scale, ++).

TMT was tested at the doses of 1.2×10^{-7}, 1.2×10^{-6}, 1.2×10^{-5} and 4.8×10^{-5} mol (Fig. 2B). At both 1.2×10^{-7} and 1.2×10^{-6} mol doses, it did not affect RT ($AUC_{0-24\,h}$ TMT vs control, NS, $n = 4$/dose). At the latter two doses, however, it induced a sustained hyperthermia with similar time-courses. The increase in RT peaked at 300 min with a value of 1.0 ± 0.3°C at the 1.2×10^{-5} mol dose ($AUC_{0-24\,h}$ TMT vs control, $P < 0.01$, $n = 4$) and at 325 min with a value of 1.0 ± 0.1°C at the highest dose ($AUC_{0-24\,h}$ TMT vs control, $P < 0.01$, $n = 4$). Furthermore, at this dose, EST decreased regularly peaking with -5.8 ± 2.0°C at 275 min. RT regained basal values after 24 h. At the highest dose tested, TMT induced moderate changes of GMB consisting in alertness and increased panting rate (arbitrary scale, ++).

Effects of co-administration of THIP and EOS on RT and GMB

THIP (6.0×10^{-8} mol, n = 4) induced a long-lasting hypothermia with a -ΔRTmax value of 1.1 ± 0.4°C at 180 min (Fig. 3A). Subsequently, RT returned to basal values at 360 min. During the hypothermic phase, sedation and motor incoordination (arbitrary scale, −−) were observed.

EOS, injected at a dose that significantly induced hyperthermia, i.e. 4.8×10^{-5} mol, partially antagonized the hypothermic effect induced by THIP. As reported in Fig. 3A, in fact, after the co-administration of the two compounds, RT decreased in the first 125 min post treatment by -0.5 ± 0.4°C, and rose slowly thereafter, peaking with 0.4 ± 0.3°C at the end of the experimental session ($AUC_{0-24\,h}$ vs THIP alone, $P < 0.05$, n = 4). After

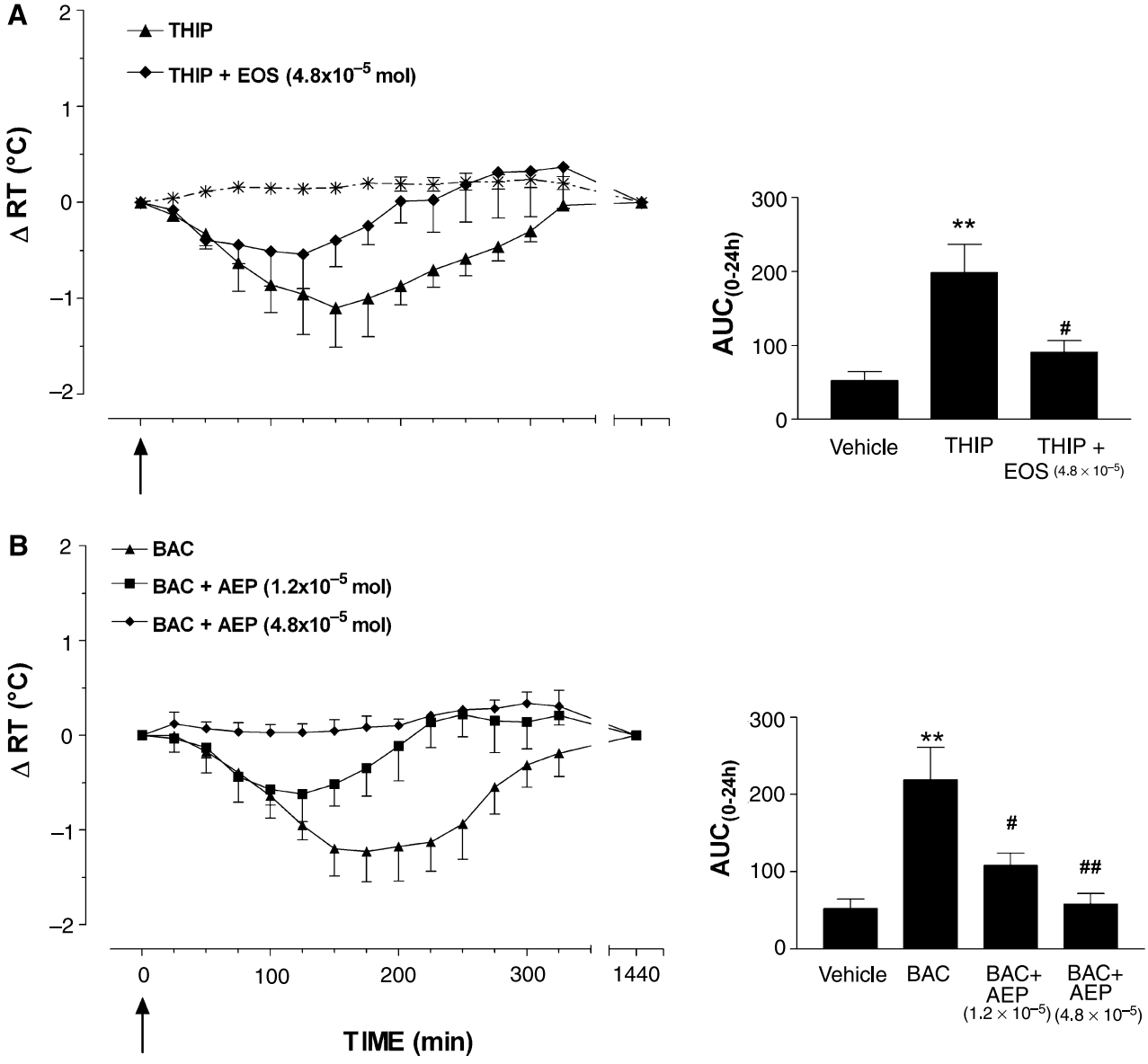

Fig. 3. **A** Effects of EOS (**A**) or Aep (**B**) on hypothermia induced by THIP and R(-)Baclofen, respectively. EOS and AEP (4.8×10^{-5} mol) were injected 10 min before THIP (6.0×10^{-8} mol, n = 4) or R(-)Baclofen (8.0×10^{-9} mol, n = 6). The hypothermia elicited by THIP or R(-)Baclofen injected alone is also reported. Data are reported as mean ± s.e.mean of changes in rabbit RT and arrows indicate the time of i.c.v. injection. In the right panels the area under the experimental curve ($AUC_{(0-24\,h)}$) of RT changes relative to the different treatments is shown. The comparison between $AUC_{(0-24\,h)}$'s was performed by using Student's t test . $^{**}P < 0.01$ vs vehicle. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs THIP or R(-)Baclofen alone

24 h RT regained basal values. During the entire experimental session, GBM was comparable to that of control animals.

R(-)Baclofen (8.0×10^{-9} mol, n = 6, Fig. 3B) caused a progressive decrease of RT with a -ΔTmax value of 1.2 ± 0.3°C at 105 min. RT regained basal values at 300 min. After R(-)Baclofen treatment, rabbits exhibited sedation and long lasting motor incoordination (arbitrary scale, − − −). At the end of the experimental session, however, GMB had returned to the normal pattern.

AEP antagonized the hypothermic and muscle relaxant effects of R(-)Baclofen in a dose-dependent fashion. The injection of 1.2×10^{-5} mol of AEP together with 8.0×10^{-9} mol of R(-)Baclofen (n = 4), partially counteracted the decrease in RT (Fig. 3B) and GMB depression (arbitrary scale, −), while at the dose of 4.8×10^{-5} mol AEP fully antagonized R(-)Baclofen-induced hypothermia (n = 4). GMB was only slightly affected by this combined treatment.

Effects of co-administration of R(-)Baclofen with DMT or TMT on RT and GMB

DMT significantly antagonized R(-)Baclofen-induced hypothermia (Fig. 4A). RT, in fact, decreased slightly after the co-administration of 1.2×10^{-5} mol of

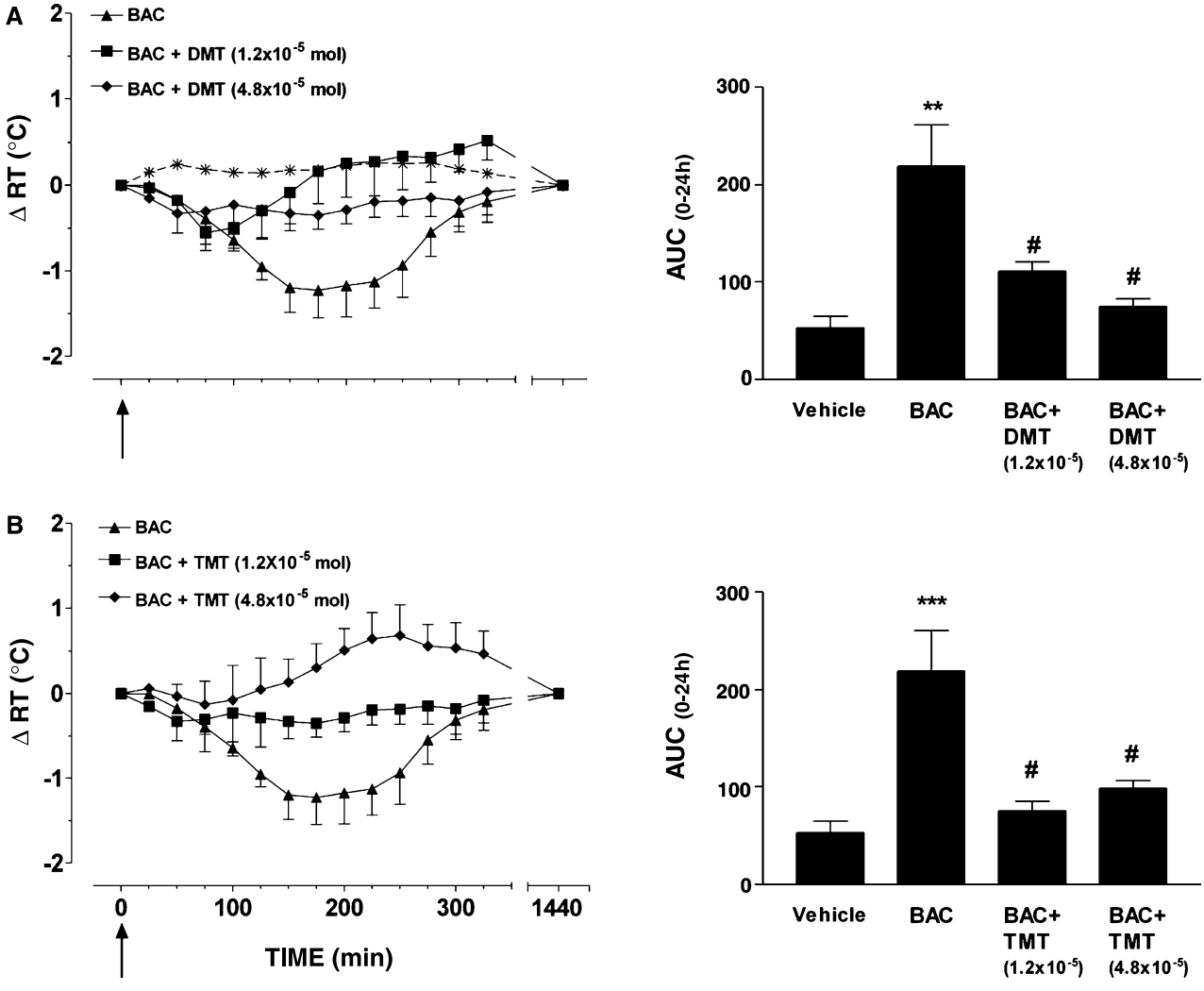

Fig. 4. Effects of DMT (**A**) or TMT (**B**) on R(-)Baclofen-induced hypothermia. DMT and TMT (4.8×10^{-5} mol, n = 4) were injected 10 min before R(-)Baclofen (8.0×10^{-9} mol, n = 6). The hypothermia elicited by R(-)Baclofen injected alone is also depicted. Data are reported as mean ± s.e.mean of changes in rabbit RT and arrows indicate the time of i.c.v. injection. In the right panels the area under the experimental curve ($AUC_{(0-24\,h)}$) of RT changes relative to the different treatments is shown. The comparison between $AUC_{(0-24\,h)}$'s was performed by using Student's t test. $^{**}P < 0.01$, $^{***}P < 0.001$ vs vehicle. $^{\#}P < 0.05$ vs R(-)Baclofen alone

DMT + 8.0×10^{-9} mol of R(-)Baclofen (n = 4), attaining a -ΔRTmax value of 0.5 ± 0.2°C at 75 min. The recovery of RT to baseline values was reached within 200 min and remained stable around the mean value of 0.3–0.4°C until the end of the experimental session. The DMT dose of 4.8×10^{-5} mol coadministered with R(-)Baclofen (n = 4), did not modify significantly RT, which remained stable and slightly lower as compared to baseline values for the entire experimental session. DMT either at 1.2×10^{-5} or 4.8×10^{-5} mol dose was able to antagonize R(-)Baclofen-induced depression of GMB, which was comparable to that of control rabbits.

TMT exhibited the same ability of DMT in antagonizing R(-)Baclofen-induced hypothermia. In particular, either at the doses of 1.2×10^{-5} or 4.8×10^{-5} mol it fully antagonized the effects of R(-)Baclofen on RT and GMB. Both doses, in fact, significantly reduced the $AUC_{(0-24\,h)}$ value of RT measured after R(-)Baclofen alone ($P < 0.05$, n = 4). In particular, the lower TMT dose did not affect RT, that remained around basal values during the entire experimental session, while the higher one produced a slight hyperthermia that peaked with a ΔRTmax value of 0.7 ± 0.3°C after 250 min and decreased slowly thereafter. After 24 h, however, RT had regained basal values.

Discussion

The aim of the present work was to verify whether some taurine derivatives, which interact with GABA-ergic system, namely EOS, DMT, TMT, AEP and PSA, are able to modify rabbit RT and GMB. The fact that these compounds interact with the GABA-ergic system, in fact, is not sufficient to predict their effects on thermoregulation as shown by the finding that β-alanine did not affect thermoregulation in the rabbit in spite of its [^{3}H]GABA displacing activity at micromolar concentrations from $GABA_B$ receptors in brain synaptic membrane preparations (Frosini et al., 2003a).

The present results demonstrate that PSA induces hypothermia accompanied by inhibition of GMB. These effects can be ascribed to the ability of this compound to slow-down the degradation of cytosolic GABA (Frosini et al., 2003a) and, consequently, to potentiate indirectly GABA effects at its post-synaptic receptors. It is well established, in fact, that the GABA-T inhibitor vigabatrin induces hypothermia (Ginefri-Gayet and Gayet, 1993) by raising brain GABA concentrations (Errante et al., 2002). Coherently, PSA has been shown in this laboratory to increase significantly GABA content in rabbit brain slices (manuscript in preparation).

It is generally accepted that the increase of GABA-ergic transmission represents a beneficial therapeutic approach to acute brain ischaemia by antagonizing excessive glutamatergic excitation responsible for cell death and, concurrently, can promote hypothermia that is ''per se'' neuroprotective, as shown in experimental as well as clinical settings (for a review see Olsen et al., 2003). The mechanisms of neuroprotection afforded by hypothermia are multifold and entail decrease of excitotoxic transmitters (e.g. glutamate and dopamine) (Busto et al., 1989), suppression of reactive oxygen and nitrogen species (Kil et al., 1996; Horiguchi et al., 2003) and release of cytochrome c (Zhao et al., 2004; Zhu et al., 2004) and caspase-3 activity (Phanithi et al., 2000). Body temperature is directly related to stroke severity and outcome, and fever after stroke is associated with substantial increase in morbidity and mortality. Body temperature control in stroke patients by administration of antipyretics during the acute phase is generally recommended, although there is no direct evidence of their temperature lowering effectiveness or their influence on the outcome of patients (Olsen et al., 2003). For this reason, novel compounds able to induce rapidly and safely hypothermia, can be very helpful in combating ischaemic brain injury. In this regard, PSA, in consideration of its ability to lower body temperature by increasing GABA-ergic tone, can be an useful lead compound for the design of novel drugs which protect the brain against ischaemic injury. Moreover, some authors have ascribed neuroprotection afforded by GABA agonists exclusively to their ability to induce hypothermia (Kuhmonen et al., 2002). Recent *in vitro* studies have provided insights into the mechanisms of neuroprotection by these compounds. When using combined oxygen and glucose deprivation as a model of *in vitro* ischaemia, the activation of GABA-ergic system was shown to provide neuroprotection (Galeffi et al., 2000; Bickler et al., 2003). In this context it is worth to outline that vigabatrin, which exhibits neuroprotective activity in a gerbil model of repetitive forebrain ischaemia (Shuaib et al., 1992, 1996), was shown to prevent ischaemia-induced field potential loss in rat corticostriatal slices. Nevertheless, the co-activation of both $GABA_A$ and $GABA_B$ receptors was required to achieve vigabatrin-(and GABA-) mediated neuroprotection during energy deprivation (Costa et al., 2004).

The present results show that AEP, EOS, DMT, TMT caused a dose-related hyperthermia accompanied, in some case, by moderate (DMT) or slight (TMT) changes in GMB. Neurochemical studies of the interaction of the above-mentioned compounds with GABAergic systems

of the rabbit (Frosini et al., 2003a) and rat (Marangolo et al., 1997) brain demonstrate that AEP, DMT, TMT and EOS possess an affinity in the micromolar range for $GABA_B$- and $GABA_A$-receptors, respectively, in whole brain synaptosomal membrane preparations. It is interesting that EOS, despite its ability to inhibit rat and mouse brain GABA transaminase (Phillips and Fowler, 1982; Qume and Flower, 1997) was found to be ineffective towards the rabbit brain enzyme (Frosini et al., 2003a). In order to further investigate the possibility that the effects of these taurine derivatives on body temperature is mediated by their interaction with GABA receptors, their ability to counteract hypothermia induced by $GABA_A$ or $GABA_B$ agonists has been assessed. In particular, THIP and R(-)Baclofen were injected at different amounts so that comparable hypothermic effects were obtained (Frosini et al., 2004). EOS antagonized in a dose-related fashion hypothermia induced by THIP, while AEP, DMT and TMT antagonized that induced by R(-)Baclofen. These results, along the above-mentioned binding data, strongly suggest that EOS behaves like a $GABA_A$ antagonist while AEP, DMT and TMT like $GABA_B$ antagonists in the central mechanisms of rabbit thermoregulation.

In recent years a number of $GABA_B$ antagonist have been developed. Some of them are structurally related to baclofen and have been designed by bioisosteric replacement of the carboxyl group of baclofen with phosphonic or sulfonic acid groups (i.e. phaclofen and saclofen, respectively) (Krogsgaard-Larsen, 1988). Omologues of (3-aminopropyl)ethylphosphinic acid represent novel, potent and orally active compounds with a nanomolar affinity for $GABA_B$ receptors. By analyzing the molecular structures and conformations of the compounds developed so far, it seems that the substitution of GABA-carboxyl group with the afore-mentioned moieties shifts GABA-biological activity, giving rise to $GABA_B$ antagonists. However, all phosphinic derivatives possess both a 3 C chain length as well as a free amine group. The present results, however, indicate that DMT and TMT behave like $GABA_B$ antagonists in the central pathways that regulate rabbit body temperature thus indicating that also carbon chain length and amine group are crucial. Furthermore, when the hydrogens of N are replaced by bulky groups, the resulting compounds are not active, suggesting that also the steric hyndrance on the N atom is important (Frosini et al., 2003a, 2003b). Finally, to explain why AEP, DMT, TMT and EOS elicited hyperthermia, we can postulate that they are able to block $GABA_A$ or $GABA_B$ receptors without activating them and thus preventing endogenous GABA from exerting a tonic influence on thermoregulatory centers that are directed towards dissipation of body heat. Without this influence the system will be unbalanced and hyperthermia will develop.

In conclusion, the pharmacological increase of central GABA-ergic tone results in a consistent decrease in RT along with depression of GMB, inhibition of muscle tone and synchronization of the ECoG signal recorded from sensorimotor cortex (Turski et al., 1990, Frosini et al., 2004). It has been hypothesized that the muscle-relaxant properties of GABA and $GABA_A$/$GABA_B$ agonists might depend on the inhibition of neuronal activity at sensorimotor cortex. Consequently, the hypothermogenic effect elicited by these compounds might be partially due – besides their effects on hypothalamic nuclei of thermoregulation – to the inhibition of shivering and basal muscular thermogenesis. While $GABA_B$ antagonist generally do not affect motor behaviour (Phillis et al., 2001; Frosini et al., 2004) $GABA_A$ antagonists promote excitation of GMB along with hyperthermia. In the present experimental protocol, however, EOS did not affect GMB, while it behaved like a $GABA_A$ antagonists in the central mechanisms of thermoregulation. This pharmacological separation of the effects on body temperature from those on GMB suggests the involvement of different $GABA_A$ receptor subunit(s) or hypothetical receptor subtypes.

Acknowledgement

This work was supported by University of Siena, PAR Progetti.

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