THE ROLE PLAYED BY ENVIRONMENTAL TEMPERATURE ON WORSENING EFFECTS OF SINGLE INJECTION OF (±)CIS-4,4'DMAR, (±)TRANS-4,4'DMAR AND THEIR CO-ADMINISTRATION IN MICE

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Introduction: The trend on synthetic drugs has been changed worldwide in the last few years. In particular, the numbers and types of Novel Psychoactive Substances (NPS) increased constantly causing intoxications and fatalities. Among the NPS, a novel synthetic stimulant called "4,4'-Dimethylaminorex (4,4'-DMAR)" has been detected by custom authorities for the first time in the Netherlands. It has been considered as a derivative of two different stimulants: Aminorex and 4-methylaminorex (4-MAR). The presence of two chiral centres in oxazoline ring in the molecular structure of 4,4'-DMAR gives it racemic properties. It has been reported on Internet different undesired effects of racemic mixture of 4,4'-DMAR in human including nausea, agitation, hyperthermia, seizures and an increase of heart rates that could be involved in cases of cardiac arrest. In particular, hyperthermia is one of the main dangerous adverse effects caused by stimulant drugs and it may be worsened with the increase of environmental temperature. Therefore, we investigated the role played by environmental temperature in worsening physiological and neurological statements of mice after intraperitoneal injection of single (±)Cis 4,4'-DMAR,(±) trans 4,4'-DMAR and their co-administration.

Material and methods: Adult male ICR (CD-1®) mice were used for this study. (±) cis-4,4'-DMAR and (±) trans-4,4'-DMAR were administrated by intraperitoneal injection at a volume of 4µl/g. The doses of (±) cis-4,4'-DMAR (10 mg/kg and 60 mg/kg; i.p.), (±) trans-4,4'-DMAR (30 mg/kg; i.p.) and their co-administration (cis 10 mg/kg + Trans 30 mg/kg i.p. and cis 60 mg/kg + Trans 30 mg/kg i.p.) were chosen basing on previous studies. The effects of both compounds cis and trans 4,4'-DMAR were investigated and analyzed off-line by a different trained operator that gives test scores. All experiments were performed between 8.30 AM to 2.00 PM. We detected for 5hours the following parameters: sweating, core temperature, seizures and lethality in mice subjected to three different temperatures (T1= 22-23°C) , (T2= 26-27°C) and (T3= 32-33°C). Convulsions and lethality are expressed as frequency (percent of animals). The core temperature is expressed as the difference between control temperature (before injection) and temperature following drug administration (Δ°C). It was detected every hour after the injection by a probe (1mm diameter) that was gently inserted, after lubrification with liquid Vaseline, into the rectum of the mouse (to about 2cm) and left in position until stabilization of the temperature (about 10 seconds). The probe was connected to a Cole Parmer digital thermometer (model 8402). Sweating is simply reported if it's present or not in mice.

Results: Systemic administration of (±) cis-4,4'-DMAR (10 mg/kg and 60 mg/kg; i.p.) at T1(22-23°C) induced sweating in mice only at highest dose (60 mg/kg). Differently, at T2(26-27°C) and T3(32-33°C) (±) cis-4,4'-DMAR induced sweating in a dose-dependent manner. Vehicle injection did not change the basal core temperature (37.2± 0.5) after 30 minutes from drug injection (Δ= -0.3°C). At T14,4'-DMAR ineffective at 10 mg/kg (Δ= 0.4°C); at 60 mg/kg increased core temperature (Δ= 2.2°C). At T2and T34,4'-DMAR injection provoked seizures and mice died in a short time period after injection. Systemic administration of (±) trans-4,4'-DMAR (30 mg/kg; i.p.) does not induce sweating, seizure and death in mice at all temperature tested. Co-administration of 4,4'-DMAR at both dosages (cis 10 mg/kg + Trans 30 mg/kg i.p) and (cis 60 mg/kg + Trans 30 mg/kg i.p.) induced sweating in mice at all temperature tested. Therefore, already at T1co-administration induced in mice a marked hyperthermia (from basal increased respectively to Δ= 1.1°C and Δ= 2.2°C). Increasing the environmental temperature (T2and T3) the effect of cis + trans 4,4'-DMAR involve increasing of frequency of seizures in mice and consequently increased fatalities in all mice tested.

Discussion and conclusion: The present study showed the importance of environmental temperature in the toxicity induced by the single (±) cis-4,4'-DMAR (10 mg/kg and 60 mg/kg; i.p.) and its co-administration with (±) trans-4,4'-DMAR. Indeed, (±) cis-4,4'-DMAR induced hyperthermia at all temperatures; likewise, seizures and death that were dose and environmental temperature depended. The co-administration of the two compounds cis + trans 4,4'-DMAR, synergistically potentiates these detrimental effects. In conclusion, our study demonstrates that environmental temperature plays a fundamental role in the toxicity of 4,4'-DMAR specifically the cis form and the co-application of both enantiomers, suggesting their dangerous effect on human health.