Research Article

Mu, Delta and Kappa Opioid Receptor Involvement in the Hypothermic Response to Caffeine and Theophylline

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Summary. *This study describes the effect of the methylxanthines, caffeine and theophylline, on core body temperature (T_b) of unrestrained mice at normal ambient temperature. Acute administration (IP) of caffeine and theophylline produced a dose-dependent hypothermia; caffeine induced hypothermia was of a greater magnitude and longer time-course than theophylline. The hypothermic response was attenuated (50%) by naloxone HCl, a peripheral and central non-selective opioid antagonist, showing that methylxanthine-induced hypothermia is partly mediated by opioid receptors. In part the hypothermic response appeared to be naloxone-insensitive (50%) indicating that other mechanisms may mediate this effect. Only theophylline-treated mice exhibited an attenuation by 25% of this response when pretreated with naloxone methiodide which only acts peripherally, indicating that part of the opioid receptor mediation of theophylline-induced hypothermia is dependent on a peripheral mechanism. No attenuation occurred when theophylline- and caffeine-treated mice were pretreated with low-dose naloxone HCl, a Mu- selective antagonist while naltrindole HCl, a Delta-selective antagonist produced a mild attenuation. The greater attenuation produced by nor-binaltorphimine, a Kappa-selective antagonist, would suggest that the Kappa receptors are mainly responsible for the opioid receptor mediation of both caffeine- and theophylline-induced hypothermia. The acute effects of caffeine and theophylline on thermoregulation are not unlike those of morphine and endogenous opioids. The pharmacological, clinical and biological significance of the thermoregulatory effects of caffeine and theophylline are discussed.*

Keywords: *caffeine, theophylline, naloxone, naltrindole, nor-binaltorphimine, body temperature, mouse*

Drugs exerting their effects through the opioid system have profound effects on body temperature. The particular effect seen is dependent on species, ambient temperature, degree of restraint imposed on the subject and route of drug administration (Adler et al, 1988).

Moderate to high doses of caffeine (>30mg/kg) have been reported to induce dose-related reductions in core body temperature in rats and mice (Durcan et al, 1991) kept at ambient temperatures in the standard range. Doses of caffeine in the range from 37.5mg - 300mg/70kg in stimulant drug abusers have also been reported to cause up to 4°C drop in skin temperature (Rush et al, 1995).

There are a number of reports of methylxanthines exerting their effects through interactions with opioid receptors, although the majority of studies have used caffeine. High doses of caffeine and other methylxanthines have been reported to potentiate the antinocioceptive actions of opioid receptor agonists such as morphine (Misra et al, 1985; Sweeny et al, 1991; Nicholson et al, 1991). Narcotic administration particularly of morphine sulphate and fentanyl can be safely carried out in the preterm infant when using intravenous caffeine simultaneously to offset the risk of apnea (Mainous, 1995).

It has been reported that naloxone hydrochloride (a non-selective opioid antagonist) attenuates the hypothermic action of caffeine (Durcan et al, 1992). The attenuation of the hypothermic effect should apply to other methylxanthines provided that the dose of naloxone used does not have an intrinsic hypothermic effect. Further studies are thus needed to fully define the role, precise mechanisms and the extent of interaction.

At present there are no studies on the involvement of the main opioid receptor types (i.e., *mu, delta* or *kappa*) in the hypothermic effect caused by methylxanthines. Discrimination between the individual involvement of each opioid receptor type would enable the determination of the exact molecular target of methylxanthines as well as to determine the involvement of central and/or peripheral effects.

In this series of experiments reported here we examined whether the hypothermic effects of caffeine and theophylline are opioid-dependent, receptor type specific and peripheral or central in origin with respect to the opioid system.

Materials and methods

Subjects

Twenty male albino mice (inbred), 8 weeks old, weighing 24g - 34g at the start of the experiment were used. They were singly housed on a 12:12 hr light:dark cycle with food and water available *ad libitum* except during testing. The mice were handled daily to reduce stress trauma to a minimum.

Temperature studies were carried out in the animal house

(temperature 20 ± 1oC and 60% ± 5% relative humidity). Experiments were performed between 10.00 hrs and 17.00 hrs and were carried out every 4 days to ensure wash out and complete metabolism of the drug.

Before the experiments were initiated, the mice were acclimatised to the surrounding environment for a period of 15 days. On each experimental day, food was removed from the cage (but not water). Food consisted of sunflower seeds, wheat, green rice, peas, sorghum seeds and green pellets mainly made up of dehydrated alfalfa meal (Kik Rico, Encia, Italy).

Temperature Recording

Core body temperature was measured using a rectal thermistor probe for mice and a digital thermometer (Physitemp Inc. formerly Sensortek Inc. New Jersey, USA). The probe was inserted 2.5 cm into the colon of each mouse.

During temperature measurements, the mice were unrestrained and were simply held gently at the base of the tail. A baseline temperature was taken before the animals were treated.

Procedure

In PART I of the study 2 groups of mice (N = 10), either received caffeine or theophylline. Animals were injected intraperitoneally (i.p.). 5 doses of caffeine and theophylline were selected: vehicle, 30mg/kg, 60mg/kg, 90mg/kg and 120mg/kg. These doses were administered in a couterbalanced order. Caffeine and theophylline (aminophylline) were dissolved in distilled water and then warmed for complete dissolution.

Colonic temperature of each mouse was taken every 2 minutes in the injecting sequence such that 20 minutes would have elapsed between injection and the first temperature recording. Colonic temperatures were recorded every 20 minutes for each mouse for a period of 140 minutes post drug administration.

In PART II of the study 2 groups of mice (N = 10), received a fixed dose of naloxone followed by either a fixed dose of caffeine or theophylline. From Part I of the study it was determined that 90 mg/kg caffeine and 120 mg/kg theophylline would be used as the fixed dose. 3 mg/kg naloxone was selected on the basis of previous reports in the literature (Durcan et al, 1992). 1 mg/kg naloxone methiodide, injected subcutaneously, was also selected on the basis of previous reports in the literature (Bhandari et al, 1992).

Each mouse received the following combinations in a counterbalanced order: Vehicle followed by vehicle, Vehicle followed by caffeine/theophylline, Naloxone HCl followed by vehicle, Naloxone HCl followed by caffeine/theophylline, Naloxone MeI followed by vehicle or Naloxone MeI followed by caffeine/theophylline.

Each mouse received 2 injections. Treatment sequence adopted was such that each treatment group (caffeine or theophylline) received their 2 administrations separated by a lag time of 15 minutes. 20 minutes following the second injection temperature recording (T_{20}) was started according to the injecting sequence. Prior to each injection a baseline colonic temperature was recorded.

In PART III of the study 2 groups of mice (N = 10), received a fixed dose of a selective opioid receptor antagonist followed by either a fixed dose of caffeine or theophylline. 90 mg/kg caffeine and 120 mg/kg theophylline were selected as the fixed dose. 0.3 mg/kg Naloxone hydrochloride (low dose), a selective *mu* opioid receptor antagonist was administered intraperitoneally (i.p.). The dose and route of administration was selected on the basis of previous reports in the literature (Kamei et al, 1992). 10.0 mg/kg Naltrindole hydrochloride, a selective *delta* opioid receptor antagonist was administered intraperitoneally (i.p.). The dose and route of administration was selected on the basis of previous reports in the literature (Toyoshi et al, 1992). 5.0 mg/kg Nor-Binaltorphimine, a selective *kappa* opioid receptor antagonist was administered subcutaneously (s.c.) in the nape of the neck. The dose and route of administration was selected on the basis of previous reports in the literature (Suzuki et al, 1992; Endoh et al, 1992).

Each mouse received the following combinations in a counterbalanced order: Vehicle followed by vehicle, vehicle followed by caffeine/theophylline, Naloxone HCl followed by vehicle, Naloxone HCl followed by caffeine/theophylline, Naltrindole HCl followed by vehicle, Naltrindole HCl followed by caffeine/ theophylline, Nor-binaltorphimine followed by vehicle, Nor-binaltorphimine followed by caffeine/ theophylline.

Each mouse received 2 injections. The lag time between the first and second treatment (I2) was 15 minutes for naloxone hydrochloride and 15 minutes for Naltrindole hydrochloride (based on previous reports by Toyoshi et al, 1992), 2 hours for Nor-Binaltorphimine (based on previous reports by Suzuki et al, 1992; Endoh et al, 1992). 20 minutes following the second injection temperature recording (T_{20}) was started according to the injecting sequence. Prior to each injection a baseline colonic temperature was recorded.

Drugs

Anhydrous caffeine (Sigma Chem. Co., Poole, UK), Theophylline as aminophylline (Sigma Chem. Co., Poole, UK), Naloxone hydrochloride (Sigma Chem. Co., Poole, UK), Naloxone methiodide (Research Biochemicals Inc., Natick, USA), Nor-Binaltorphimine (Research Biochemicals Inc., Natick, USA), Naltrindole hydrochloride (Research Biochemicals Inc., Natick, USA).

Statistics and Analysis

Data were analysed using analysis of variance (ANOVA) supplemented where appropriate by tests of simple main effects. A two way analysis with drug/drug dose and time as within subject variables were used for the three studies. All data are shown as means of change in temperature from baseline at 20 minute intervals for 140 minutes.

Results

Acute effects of caffeine on body temperature

Caffeine, produced a dose-dependent decrease in body temperature which followed different time-courses, main effects of dose [F(4, 36) = 11.93, p<0.001], and time, [F(7, 63) = 37.20, P<0.001], and the dose x time interaction, [F(28, 252) = 6.76, P<0.001].

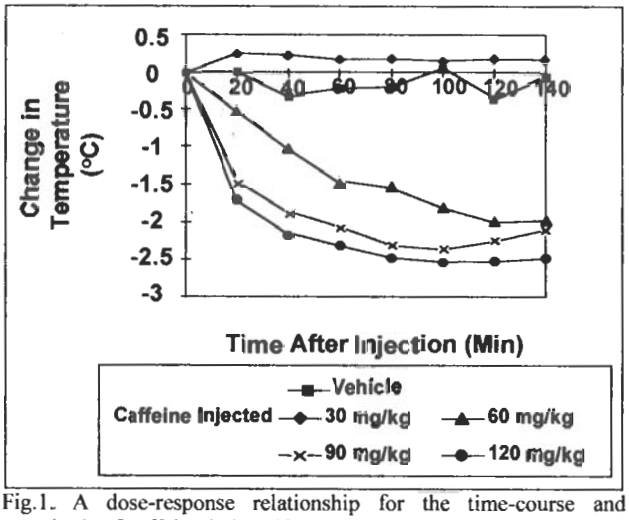

Fig.1. A dose-response relationship for the time-course and magnitude of caffeine-induced hypothermia in mice,

The onset of the caffeine-induced hypothermic effect was dose dependent. The 60 mg/kg induced an initial (20 minutes) drop in body temperature of around 0.52°C, [F(1, 315) = 4.46, p<0.050, from baseline, but was not statistically significant [F(1,288) = 1.30, p>0.100]. However, the drop in body temperature continued to develop over the test period reaching a peak change in temperature of 2.00°C below baseline at 120 minutes post caffeine administration, [F(1, 315) = 65.91, p<0.001].

The hypothermic response to the highest doses of caffeine, 90 and 120 mg/kg, were observed soon after drug administration, with a significant mean drop of 1.44°C, [F(1, 315) = 34.17, p<0.001] and 1.69°C, F(1, 315) = 47.06, p<0.001] respectively, 20 minutes post caffeine administration.

Acute effects of theophylline on body temperature

Theophylline, also produced a dose-dependent decrease in body temperature which however followed different time-courses, main effects of dose [F(4, 36) = 9.32, p<0.001], and time, [F(7, 63) = 13.35, P<0.001], and the dose x time interaction, [F(28, 252) = 5.76, P<0.001]. Doses of 60 mg/kg and above resulted in a biphasic response, that is, a period of temperature drop followed by a rise in temperature back towards baseline levels.

60 mg/kg induced an initial drop in body temperature by 0.88°C, [F(1, 315) = 12.91, p<0.001], below baseline, 20 minutes post theophylline administration. This initial drop in core body temperature was of short duration and coincided with the peak drop in body temperature.

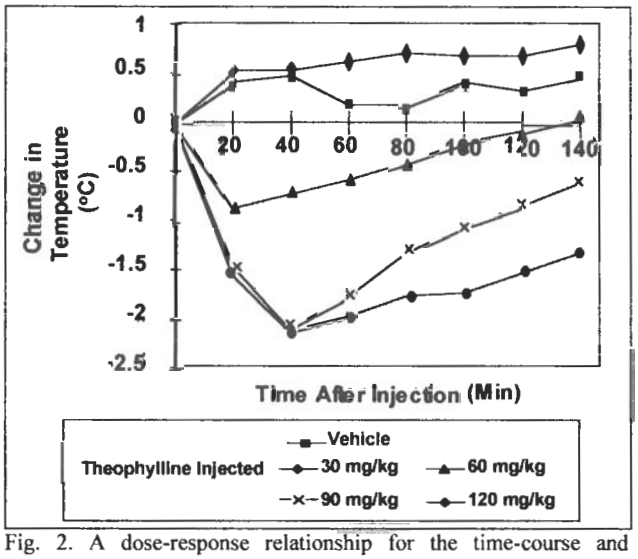

Fig. 2. A dose-response relationship for the time-course and magnitude of theophylline-induced hypothermia in mice

Following the initial temperature drop, core body temperature gradually but consistently approached baseline levels. At 100 minutes post theophylline administration no statisticaly significant effect was found [F(1, 315) = 0.81, p>0.100].

The hypothermic response following the highest doses of theophylline, 90 and 120 mg/kg, were observed soon after drug administration. At 20 minutes, 90 mg/kg theophylline produced a mean drop of 1.44°C below baseline, [F(1, 315) = 34.57, p<0.001].

Naloxone effects on caffeine-induced hypothermia.

Pretreatment with the centrally- and peripherally-acting non-selective opioid antagonist, naloxone hydrochloride, at a dose of 3 mg/kg attenuated the hypothermic action of 90 mg/kg caffeine as reflected by an upward shift in the temperature-time chart.

The attenuation was by approximately 1.23°C as reflected by the magnitude of the hypothermic effect at 100 minutes (time of peak hypothermia) post caffeine administration, [F(1, 378) = 42.23, p<0.001]. This temperature elevation was however significantly lower than that of naloxone HCl-pretreated, vehicle-treated mice, [F(1, 360) = 23.58, p<0.001].

The significance of this attenuation can be seen when contrasted with the temperature drop (at 100 minutes) of the vehicle-pretreated, caffeine-treated mice (2.29°C) and that of naloxone HCl- pretreated, caffeine-treated mice (1.23°C), [F(1, 360) = 11.94, p<0.001]. The attenuation at peak hypothermia was not the most pronounced. Attenuation was greatest at 20 minutes post caffeine administration, reaching, 0.7°C above that of the caffeine-treated mice.

Pretreatment with the peripherally-acting non-selective opioid antagonist naloxone methiodide, at a dose of 1 mg/kg did not attenuate the hypothermic action of 90 mg/kg caffeine. Naloxone methiodide-pretreated, caffeine-treated mice showed a larger drop below baseline

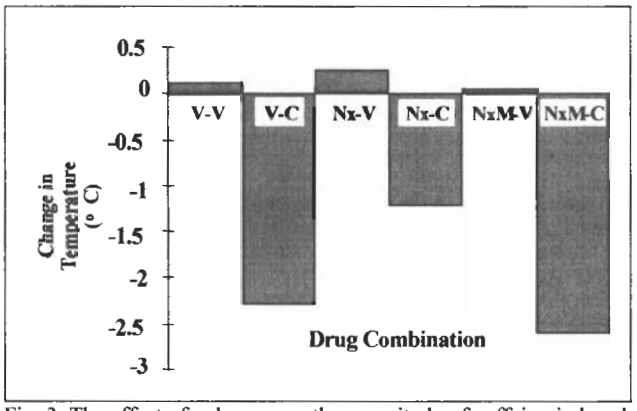

Fig. 3. The effect of naloxone on the magnitude of caffeine-induced hypothermia with time. Vehicle-vehicle (V), vehicle-caffeine (VC), naloxone-vehicle (NxV), naloxone-caffeine (NxC), naloxone methiodide-vehicle (NxMV), naloxone methiodide-caffeine (NxMC), *** P<0.001.

but the difference between the two combinations was not significant as demonstrated at 100 minutes post caffeine administration, [F(1, 360) = 1.02, p>0.100].

At the doses used neither Naloxone methiodide (1 mg/kg) nor Naloxone HCl (3 mg/kg) produced an intrinsic effect on core body temperature.

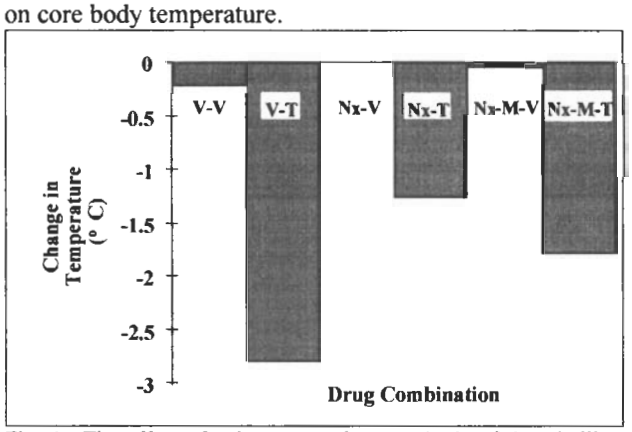

Fig. 4. The effect of naloxone on the magnitude of theophylline-induced hypothermia with time. Vehicle-vehicle (VV), vehicle-theophylline (VT), naloxone-vehicle (NxV), naloxone-theophylline (NxT), naloxone methiodide-vehicle (NxMV), naloxone methiodide-theophylline (NxMT), *** P<0.001.

Naloxone effects on theophylline-induced hypothermia

Pretreatment with the centrally- and peripherally-acting non-selective opioid antagonist, naloxone hydrochloride, at a dose of 3 mg/kg attenuated the hypothermic action of 120 mg/kg theophylline as reflected by an upward shift in the temperature-time chart.

The 120 mg/kg theophylline-induced hypothermia in mice pretreated with naloxone hydrochloride was significantly attenuated by approximately 50% as reflected by the magnitude of the hypothermic effect at 60 minutes post theophylline administration [F(1, 378) = 26.97, p<0.001].

Pretreatment with the peripherally-acting non-selective opioid antagonist, naloxone methiodide, at a dose of 1 mg/kg attenuated the hypothermic action of 120 mg/kg theophylline.

The 120 mg/kg theophylline-induced hypothermia in mice pretreated with naloxone methiodide was significantly attenuated by about 35% as reflected by the magnitude of the hypothermic effect at 60 minutes post theophylline administration (time of peak hypothermia). Temperature attenuation was of the order of 1.80°C.

Neither, naloxone hydrochloride nor naloxone methiodide pretreatment disrupted the biphasic temperature-time profile of 120 mg/kg theophylline.

Opioid selective antagonist effects on caffeine-induced hypothermia.

Pretreatment with naloxone hydrochloride (0.3 mg/kg), acting as the *mu*-selective opioid antagonist did not result in any attenuation of the hypothermic action of 90 mg/kg caffeine during the 140 minutes test period [F,max(1, 504) = 2.14, p>0.100].

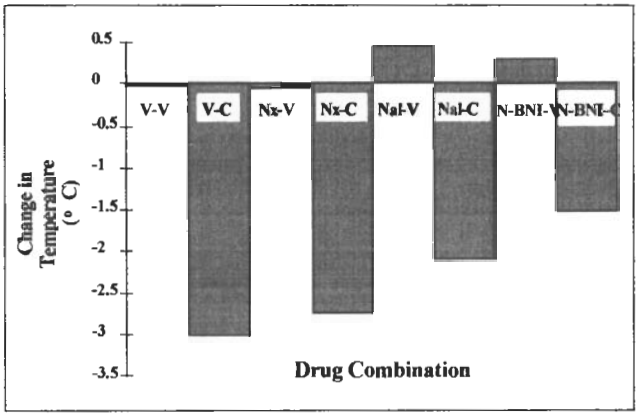

Fig. 5. The effect of selective opioid antagonists on the magnitude of caffeine-induced hypothermia with time. Vehicle-vehicle (VV), vehicle-caffeine (VC), naloxone-vehicle (NxV), naloxone-caffeine (NxC), naltrindole-vehicle (NalV), naltrindole-caffeine (NalC), nor-binaltrophimine-vehicle (NBNIV), nor-binaltrophimine-caffeine (NBNIC), *** P<0.001.

Pretreatment with 10 mg/kg naltrindole hydrochloride (the *delta*-selective opioid antagonist) resulted in an attenuation of the hypothermic effect of 90 mg/kg caffeine. This was reflected by an upward shift in the temperature-time chart.

The 90 mg/kg caffeine-induced hypothermia in mice pretreated with naltrindole hydrochloride (60 - 140 minutes post caffeine administration) was attenuated by about 30% as reflected by the magnitude of the hypothermic effect at 100 minutes (maximum attenuation) post caffeine administration [F(1, 504) = 7.41, p>0.010].

Pretreatment with 5 mg/kg Nor-binaltorphimine dihydrochloride (Nor-BNI), *kappa*-selective opioid antagonist, resulted in an attenuation of the hypothermic effect of 90 mg/kg caffeine as reflected by an upward shift in the temperature-time chart.

The difference between the two combinations was significant throughout the 140 minutes test period, [Fmax(1, 504) = 21.95, p<0.005].

The 90 mg/kg caffeine-induced hypothermia in mice pretreated with nor-BNI was (40 - 140 minutes post

caffeine administration) below baseline and was significantly attenuated.

The attenuated temperature at peak hypothermia that is, at 100 minutes post caffeine administration was 1.52°C below baseline, [F(1, 504) = 50.13, p<0.001]. This temperature was also significant when compared to that of nor-binaltorphimine-pretreated, vehicle- treated mice, [F(1, 504) = 28.40, p<0.001].

Opioid selective antagonist effects on theophylline-induced hypothermia.

Pretreatment with low dose naloxone hydrochloride (0.3 mg/kg), as the *mu*-selective opioid antagonist, resulted in no attenuation of the hypothermic action of 120 mg/kg theophylline.

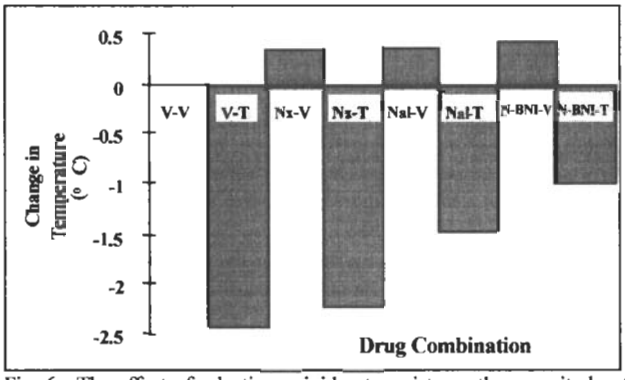

Fig. 6. The effect of selective opioid antagonists on the magnitude of theophylline-induced hypothermia with time. Vehicle-vehicle (VV), vehicle-theophylline (VT), naloxone-vehicle (NxV), naloxone-theophylline (NxT), naltrindole-vehicle (NalV), naltrindole-theophylline (NalT), nor-binaltrophimine-vehicle (NBINV), nor-binaltrophimine-theophylline (NBINT), *** P<0.001.

Although, the temperature-time profile of low dose naloxone hydrochloride/theophylline combination was above that of the theophylline-treated mice, the magnitude of the hypothermia of the two combinations was not significantly different, [Fmax(1, 504) = 1.91, p>0.100] throughout the 140 minutes test period.

Pretreatment with 10 mg/kg naltrindole hydrochloride, *delta*-selective opioid antagonist, resulted in an attenuation of the hypothermic effect of 120 mg/kg theophylline as reflected by an upward shift in the temperature-time chart.

Attenuation at peak hypothermia (60 minutes post theophylline administration) was by 39%, the attenuated temperature at 60 minutes being 1.46°C below baseline, [F(1, 504) = 44.05, p<0.001]. The significance of the attenuation was evidenced by the contrast of T_b at 60 minutes, of naltrindole/theophylline combination (1.46°C) and that of theophylline-only treated mice (2.41°C), [F(1, 504) = 7.34, p<0.010].

Pretreatment with 5 mg/kg Nor-binaltorphimine dihydrochloride (Nor-BNI) *kappa*- selective opioid selective antagonist, resulted in an attenuation of the hypothermic effect of 120 mg/kg theophylline as reflected by an upward shift in the temperature-time curve.

The 120 mg/kg theophylline-induced hypothermia in mice pretreated with nor-BNI was below baseline throughout the 140 minutes test period and was significantly attenuated by a maximum of 40% occurring at 60 minutes [F(1, 504) = 16.98, p<0.001] (maximum attenuation) post theophylline administration and a minumum of 26% occurring at 20 minutes post theophylline administration [F(1, 504) = 10.72, p>0.005].

Discussion

The results of the present study demonstrate that caffeine and theophylline, induce a dose-dependent hypothermia similar to that reported previously for caffeine and other alkylxanthines (Schlosberg, 1983; Spindel et al, 1983; Pohorechy et al, 1989; Kalhorn et al, 1990; Carney et al, 1985; Durcan et al, 1991). This hypothermic effect, in both high-dose caffeine and theophylline was seen to persist for greater than 2 hours post drug administration.

In the present study the animals (mice) were active throughout the testing period due to the repeated body temperature measurements.

From the results it would appear that doses of 60 mg/kg and above cause a dose-related hypothermia.

As opposed to caffeine, theophylline showed a biphasic response with respect to core body temperature. This is similar to what is observed with an acute dose of morphine. However, the drug did not change the set-point around which body temperature is maintained because the animals would have maintained the temperature at which T_b originally dropped if that were the case. The effect of caffeine should not be inferred to a change in set-point but rather a more persistent hypothermic effect than that produced by theophylline.

The biphasic response demonstrated by theophylline may be attributed to the shorter half-life of theophylline (Goodman-Gilman et al, 1991).

The effects of naloxone on caffeine- and theophylline-induced hypothermia.

The results of the present study confirm previous reports (Durcan et al, 1992) that caffeine/theophylline-induced hypothermia is partly mediated by opioid receptors as reflected by the partial attenuation of the hypothermia when the subjects were pretreated with naloxone HCl. Part of the caffeine-induced hypothermia seems to be naloxone- insensitive indicating that mechanisms other than opioid receptors are responsible. Theoretically, higher doses of naloxone could have resulted in a greater attenuation of the response but in practice this was not possible as doses of naloxone greater than 3mg/kg cause a hypothermic effect (Durcan et al, 1992).

Pretreatment with naloxone methiodide, a derivative of naloxone, which does not cross the blood-brain-barrier, resulted in a slight downward shift in the temperature-time curve of caffeine. However, the magnitude of the change in T_b was not significant when compared to the vehicle-pretreated, caffeine-treated mice.

The caffeine-induced hypothermia is thus partly mediated by central opioid receptors and it seems that there is little or no involvement of peripheral opioid receptors.

Thus, contrary to what was observed with caffeine, theophylline-induced hypothermia is partly mediated by central opioid receptors and partly by peripheral opioid receptors.

Since both caffeine and theophylline cross the blood-brain-barrier efficiently, the fact that part of the theophylline-induced hypothermia is peripherally mediated, shows that theophylline interacts with peripheral opioid receptors with greater affinity than caffeine. The shorter time-course of the hypothermia seen with theophylline may be due to this peripheral site of action, which is less efficacious than those found centrally.

Moreover, the difference with regards to peripheral versus central action of these drugs, is indicative of the finding that the conformation of opioid receptors in the CNS and the peripheral nervous system, are different. This is in accordance with various reports in the literature (Siegel et al, 1989) which differentiate between various opioid receptor subtypes, such as *mu*$_1$, *mu*$_2$ *and kappa*$_1$, *kappa*$_2$.

Although the attenuation by naloxone suggests that caffeine- and theophylline-induced hypothermia is opioid receptor mediated, a factor which must be considered is the interaction of caffeine and theophylline with the various opioid receptor types. Morover, caffeine is reported to alter with the basal tone of the opioid system suggesting that the induced hypothermia may be an indirect effect through the release of endogenous opioids such as *Beta*-endorphin (Arnold et al, 1983). Hence the use of naloxone at doses at which it is non-selective, make interpretation of results rather speculative as naloxone is reported to decrease *Beta*-endorphin release and also block caffeine-induced (Arnold et al, 1983) and possibly theophylline-induced stimulation of this release. Additionally, naloxone blocks *mu* opioid receptors to which *Beta*- endorphin is the natural ligand. *Beta*-endorphin and other opioid agonists, including dynorphin and U-50,488H have been shown to effect body temperature in rodents (Bhargava et al, 1989; Cavicchini et al, 1989; Olson et al, 1989; Spencer et al, 1989). However, it is not clear if caffeine and possibly other methylxanthines stimulate the release of endogenous opioids other than *Beta*-endorphin.

The effect of selective opioid antagonists on caffeine- and theophylline-induced hypothermia.

Regulation of body temperature by the opioid system is not clear cut, but generally it appears to be receptor type specific (Olson et al, 1992).

As in the case of morphine, selective mu, delta and kappa opioid agonists have also been reported to produce a differentiable profile of body temperature changes. DAMGO, a selective mu receptor agonist, caused a primary decrease in body temperature of restrained rats and an increase in body temperature of unrestrained rats (Spencer et al, 1988).

Low doses of both DPDPE, a selective delta receptor agonist, and U-50,488H a selective kappa receptor agonist, caused a decrease in body temperature of both restrained and unrestrained rats (Spencer et al, 1988, 1990).

In the present experiment, the contribution of opioid receptor types (i.e., the *mu*, *delta*, and *kappa* opioid receptors) was studied using selective opioid receptor antagonists. Pretreatment with low-dose naloxone HCl (0.3 mg/kg), acting selectively on *mu* opioid receptors, resulted in a non-significant upward shift in the temperature-time curve.

Thus, it appears that *mu* receptors of the CNS are not responsible for caffeine-induced hypothermia. This is in accordance with reports in the literature (Adler et al, 1988) that the *mu* receptors are associated with mechanisms of heat gain.

Although there is no evidence to date for the effect of low dose naloxone on *Beta*-endorphin release, one may exclude until proved otherwise, that low dose naloxone was able to decrease *Beta*-endorphin and hence produce the caffeine-induced and possibly theophylline-induced stimulation of this release meaning that caffeine and theophylline-induced hypothermia is unrelated to the Mu opioid receptors.

Although, the results suggest that *delta* opioid receptors might be involved in mechanisms of heat loss, these should not be interpreted as a possibility that *delta* opioid receptors are tonically active in thermoregulation, since the selective antagonist did not have a significant intrinsic effect on body temperature.

Therefore, the attenuation by naltrindole was not due to a counter thermoregulatory mechanism that masked the caffeine-induced hypothermia, but blockade of *delta* receptors and thus prevention of the access to caffeine (or endogenous opioid released as a result of caffeine stimulation) to the receptor site.

The administration of nor-binaltorphimine, resulted in a significant attenuation of caffeine-induced hypothermia. Consequently, it seems that the *kappa* opioid receptors are also involved in the hypothermic action of caffeine. This is in accordance with previous reports in the literature which associate dynorphins and *kappa* receptors with mechanisms of heat loss (Adler et al, 1988).

However, the attenuation obtained with nor-binaltorphimine was more significant than that obtained with naltrindole. Thus, the results show that although both *delta* and *kappa* receptors are involved in caffeine/theophylline-induced hypothermia, the *kappa* receptors are the major contributors to the mediation of the hypothermic effect.

The *Kappa* opioid receptor seems to be the major contributor towards the opioid receptor mediation of theophylline-induced hypothermia, with a minor involvement of *delta* opioid receptors. However, the

percentage attenuation of theophylline-induced hypothermia by nor-binaltorphimine was greater than that demonstrated with caffeine.

Chronic administration of U-50,488H (a selective *kappa* agonist) resulted in the development of tolerance to its analgesic and hypothermic effects (Reddy et al, 1992, Tejwani et al, 1992). These findings are in accordance with observations that methylxanthine-induced hypothermia resembles that of morphine with respect to chronic administration and the results of the present study which demonstrate that methylxanthine-induced hypothermia is partially mediated by kappa receptors.

In view of the fact that part of the opioid receptor mediation of theophylline-induced hypothermia is of peripheral origin, the greater attenuation in the case of theophylline is in accordance with reports (Adler et al, 1988) that a significant proportion of *kappa* opioid receptors are found primarily outside the brain. This, however, also suggests that binding to central *kappa* opioid receptors results in a more profound hypothermia as reflected by the dose-response relationships of caffeine.

The fact that naltrindole and nor-binaltorphimine caused an attenuation of the hypothermia, suggests that the attenuation caused by naloxone in Part II was not due to interference with the phosphodiesterase-inhibiting properties of caffeine and theophylline but due to blockade of the *delta* and *kappa* opioid receptors. Rather, it was the part of the hypothermia insensitive to the antagonists which could be the result of phosphodiesterase inhibition by caffeine and theophylline.

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