This article has not been copyedited and formatted. The final version may differ from this version.
JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333

Niacin-induced "flush" Involves Release of PGD_2 from Mast Cells and Serotonin from Platelets: Evidence from Human Cells *In Vitro* and an Animal Model

Dean Papaliodis, William Boucher, Duraisamy Kempuraj, Margaret Michaelian, Adams Wolfberg, Michael House and Theoharis C. Theoharides

Department of Pharmacology and Experimental Therapeutics, Molecular Immunopharmacology and Drug Discovery Laboratory (DP, WB, DK, MM, TCT) Departments of Internal Medicine (TCT) and Biochemistry (TCT), Obstetrics and Gynecology *(AW, MH), Tufts University School of Medicine, Tufts Medical Center, 136 Harrison Avenue, Boston, MA 02111*

RUNNING TITLE PAGE

a) Running title: Niacin flush involves mast cell PGD_2 and platelet serotonin

b) Address correspondence to:

Theoharis Constantin Theoharides, Ph.D., M.D.

Department of Pharmacology and Experimental Therapeutics

Tufts University School of Medicine

136 Harrison Avenue, Boston, MA 02111

Phone: (617) 636-6866

Fax: (617) 636-2456

Email: theoharis.theoharides@tufts.edu

c) Number of text pages	=	30
Number of tables	=	0
Number of figures	=	9
Number of references	=	40
Number of words in the Abstract	=	139
Number of words in the Introduction	=	362
Number of words in the Discussion	=	1273

d) Abbreviations:

Prostaglandin D_2, PGD_2; 5-hydroxytryptamine, 5-HT; histamine-1 receptor antagonists, H1rα; human, h

e) Recommended section: Cardiovascular

ABSTRACT

Niacin lowers serum cholesterol, LDL and triglycerides, while raising HDL. However, most patients experience cutaneous warmth and vasodilation (flush). Acetylsalicylic acid (ASA) can reduce this flush presumably by decreasing prostaglandin D_2 (PGD_2) release from macrophages. Here we show that methylnicotinate induces significant PGD_2 release from human mast cells and serotonin from human platelets. Intradermal injection of methylnicotinate induces rat skin vasodilation and vascular permeability. Niacin increases plasma PGD_2 and serotonin in a rat model of flush. The phenothiazine prochlorperazine, the H_1, serotonin receptor antagonist cyproheptadine, and the specific serotonin receptor-2A antagonist ketanserin inhibits niacin-induced temperature increase by 90% ($n= 5$, $p<0.05$), 90% and 50%, ($n=3$, $p<0.05$) and 85% ($n=6$, $p=0.0008$), respectively, in this animal model. These results indicate that niacin-induced flush involves both PGD_2 and serotonin, suggesting that drugs other than ASA are required to effectively inhibit niacin-induced flush.

JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

In spite of increased efforts to identify risk factors, and availability of better drugs for cardiovascular disease (CAD), deaths from CAD continue to increase (Libby, 2005). The B_3-vitamin niacin (nicotinic acid) has been repeatedly shown to improve all lipoprotein abnormalities and increase HDL levels (Carlson, 2005). Niacin together with a statin was further shown to be the most effective regimen for lowering cholesterol, triglycerides, LDL and apolipoprotein-a (Apo-a), while increasing HDL (Brown, et al., 2001). Moreover, slow release niacin combined with lovastatin was shown to have a superior lipoprotein lowering profile (Gupta and Ito, 2002). However, a limiting adverse effect in patients receiving niacin (1-2 g/day) is the development of significant cutaneous warmth and facial vasodilation, usually referred to as "flush," which limits compliance extensively (Gupta and Ito, 2002).

Niacin is thought to induce flush by stimulating the release of prostaglandin D_2 (PGD_2) from the skin (Morrow, et al., 1989;Morrow, et al., 1992). The actual cell type responsible for PGD_2 release in response to niacin is unknown, but dermal macrophages (Urade, et al., 1989) and Langerhan's cells (Benyo, et al., 2006) have been implicated. However, co-administration of acetylsalicylic acid (ASA) to reduce PGD_2 levels has not been particularly effective (only 30% inhibition) in blocking niacin flush (Jungnickel, et al., 1997;Dunn, et al., 1995), implying that molecules other than PGD_2 may be involved. These may include histamine and serotonin, which could be released from mast cells (kushnir-Sukhov, et al., 2006). Serotonin is also released from platelets (D'Souza, et al., 2006) and from enterochromaffin cells especially in Carcinoid syndrome, which commonly presents with facial flush (Loong, et al., 1968).

Here, we investigated if niacin could induce histamine and PGD_2 release from human mast cells, as well as serotonin release from human platelets. We also investigated if niacin could induce skin vasodilation and vascular permeability in rat skin. Finally, we investigated if niacin could increase plasma PGD_2 and serotonin in a rat model of niacin-induced flush (Turenne, et al., 2001) and whether serotonin receptor antagonists could inhibit this effect. Our results show that niacin-induced flush involves both PGD_2 from mast cells and serotonin from platelets. Serotonin-receptor antagonists block niacin-induced flush and could be helpful clinically.

Methods

Animals

Male Sprague-Dawley rats (300-350 g) were housed three per cage and were provided with food and water *ad libitum*. The room temperature was constant at 21±1^{o}C, with a 14:10 h light:dark schedule and lights out at 1900 h. Animals were allowed to habituate to housing conditions and were handled daily for 3 days before they were used in any experiment. Niacin, methylnicotinate, methyl nicotinamide, nicotinamide, nicotinamide-N-oxide and nicotinuric acid, as well as brompheniramine, chlorpheniramine, cyproheptadine, diphenhydramine, disodium cromoglycate (cromolyn), dipyridamole, hydroxyzine, ketanserin, ketotifen and prochlorperazine were purchased from Sigma (St. Louis, MO), while azatadine was from Schering-Plough Co. (Killinworth, NJ). All drugs were dissolved in 0.9% NaCl and were prepared fresh each day.

Assessment of niacin-induced skin temperature changes

Temperature measurements were recorded using a hand-held infrared pyrometer (Model OS613A, Omega Co., Stamford, CT) connected to a millivoltmeter. The probe was held at a distance of 1-2 mm from the animal's skin and temperature readings were taken from a circular area approximately 3 mm in diameter. Skin temperature was initially recorded from the abdominal area, the tail and the ear, but it was determined that ear temperatures gave the most reliable results. Animals were habituated to handling and to the infrared probe for 3 days before use. On the day of the experiment, the animals were brought into the lab (9-10 AM) and the ear temperature was measured. Three readings from the top half of each ear were recorded routinely for each time point and they varied less than 15%. Baseline ear temperature was recorded immediately before animals were injected intraperitoneally (ip) with either niacin or the test

drug. In no experiment was there a significant difference between baseline temperatures of each of the treatment groups. In some of the preliminary experiments, the animals were first anesthetized with a single ip injection of 0.5 ml solution containing ketamine (66 mg/kg) and xylazine (16.5 mg/kg). Ear temperature was then recorded at various intervals and another baseline value was taken immediately before administration of niacin, following which the ear temperature was then measured every 10 min for a period of 60 min. The amount of niacin used varied between 8.25 – 33 mg/kg. The dose of 24.75 mg/kg chosen for most experiments was roughly equivalent to 1,750 mg / 80 kg, the average effective dose used in humans to induce significant reductions in triglycerides and LDL. The animals were returned to their cages between measurements. Animals were "rested" for one week and were used again; the effect of niacin was not changed in rats that were used more than once. This protocol was approved by the Tufts Medical Center Animal Use Committee (Protocol # 41-05).

Pre-treatment with various drugs

Rats were randomly administered either (A) vehicle followed by niacin or (B) test drug followed by niacin. The length of pretreatment before niacin injection varied and it was recorded up to 8 hr in some cases.

Isolation of $CD34^+$ cells and mast cell culture

Human umbilical cord blood was collected as approved by the Tufts Medical Center's Human Investigation Review Board (protocol # 7305). Non-phagocytic mononuclear cells were separated by density-gradient centrifugation using Lymphocyte Separation Medium (LSM) from Organon Teknika Corp (Durham, NC). The isolation of hematopoietic stem cells ($CD34^+$) was

JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

performed by positive selection of $CD34^+/AC133^+$ cells by magnetic associated cell sorting (MACS) using an $AC133^+$ cell isolation kit (Milltenyi Biotec, Auburn, CA). hCBMCs were cultured as previously reported (Kempuraj, et al., 1999). Briefly, $CD34^+$ cells were suspended in Iscove's Modified Dulbecco's Medium (IMDM; GIBCO BRL), supplemented with 200 ng/ml rhSCF, 50 ng/ml IL-6, 5% fetal bovine serum (FBS; Biowhittaker, Walkesville, MD), $5x10^{-5}$ M 2-Mercaptoethanol, and 1% penicillin-streptomycin (GIBCO BRL) for 12 to 16 weeks. The purity of hCBMCs was evaluated by immunocytochemical staining for tryptase as previously described (Kempuraj, et al., 1999) and mast cell viability was determined by trypan blue (0.3%) exclusion.

Human platelets

Freshly isolated human platelets were purchased from Allcells (Emeryville, CA) and sent to us overnight. Platelets were used the day of arrival after washing as indicated below.

The hCBMCs or human platelets were washed once in DPBS and Human Tyrode's buffer. Cells were stimulated in Tyrode's buffer in a 37ºC shaking water bath. Then the tubes were centrifuged and the supernatant fluid was collected and stored at -80°C until assays. Histamine level was assayed from the supernatant and pellet and the percent histamine release was calculated as reported previously (Kempuraj, et al., 2005). PGD2 levels in the hCBMCs supernatant fluid was assayed by enzyme immuno assay (EIA) using commercial kit (Cayman Chemical Company, Ann Arbor, MI) as per the kit procedure (Papaliodis, et al., 2008).

Blood mediator measurements

In certain cases, plasma levels of PGD_2 and serotonin (Immunotech, France) were assayed by EIA as per the kit's procedure.

Intradermal injection and measurement of vascular permeability

Rats were anesthetized with a single ip injection of 0.3 ml ketamine (66 mg/kg) and xylazine (16.5 mg/kg) . While under anesthesia, an amount of 0.3 ml Evans blue (1%) was injected in the tail vein. Thirty min later, the test drugs (niacin, niacin metabolites, neurotensin, as a positive control) and normal saline (as a negative control) were injected (0.05 ml) intradermally at different adjacent spots on the back of the rat using tuberculin syringes at the concentrations noted in the appropriate legend (Fig. 4). At the end of the injections, the animal was kept under anesthesia for another 15 min following which it was killed by asphyxiation over CO_2 vapor and decapitated. The skin was then removed, turned over, and photographed. Any vascular permeability is noted as dye extravasation (Theoharides, et al., 1998).

Statistical analysis

The six ear temperature measurements (three from each ear) were averaged for each time point. All data from multiple rats were from separate experiments and are presented as mean± SD or percent change from that recorded after niacin administration and varied less than 15%. Any temperature change was calculated by subtracting from the mean value for each experimental time point the baseline temperature obtained immediately before the drug/vehicle was injected or the baseline measured immediately before niacin administration, which ever was appropriate. The human platelet serotonin values presented as a scattergram, with the meana group value shown by a horizontal bar, in order to show the variability from different donors.

Paired comparisons between niacin and control, or niacin and drug pretreatment followed by niacin, were analyzed with either the paired Student's t-test or the non-parametric Mann-Whitney U test; the latter was used because it was not known if the effect of niacin followed a normal distribution. Multi-variant ANOVA analysis was performed on all other comparisons. Significance is denoted by $p<0.05$.

JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Results

Effect of niacin and niacin metabolites on human mast cell and platelet secretion

Methylnicotinate had a small dose-response effect on human mast cell secretion of histamine (Fig. 1A) and an impressive dose-dependent effect on mast cell secretion of PGD_2 (Fig. 1B). Niacin, nicotinamide and nicotinuric acid had negligible effects (results not shown).

Niacin and its metabolites, nicotinamide and nicotinuric acid, as well as the niacin derivative (synthetic niacin esther) methlnicotinate (at about 0.1 mM) all induced serotonin release (Fig. 2). In fact, methylnicotinate induced human platelet secretion of serotonin (Fig. 2). These results are shown as a scattergram, including group means, to point out the different reactivity of platelets from individual platelet donors; this variability may explain the well known differences in flush severity experienced by different patients.

Effect of niacin on rat plasma PGD_2 and serotonin levels. We then investigated the effect of niacin, as well as the effect of certain test drugs on rat, serum PGD_2 and plasma serotonin. Niacin (24.75 mg/kg) increased plasma PGD_2 from 933±94 pg/ml to 1750±352 pg/ml at 45 min (n=3, p=0.018, Fig. 3A) and plasma serotonin from 137±37 ng/ml (n=4) to 260±28 ng/ml (n=4, p=0.01, Fig. 3B). There was no effect on serum histamine or PGE_2 (results not shown). Pretreatment for 2 hr with ASA (4.02 mg/kg) reduced plasma PGD_2 by 85% to 1050±212 pg/ml (n=3, p=0.018, Fig. 3A), but had no statistically significant inhibitory effect on plasma serotonin levels (Fig. 3B). Cyproheptadine (56.4 µg/kg) did not affect either plasma PGD_2 or serotonin (n=4, p=0.7023, Fig. 3B).

Effect of niacin and metabolites on rat skin vasodilation and vascular permeability. We then investigated if intradermal injection of niacin or its key metabolites could induce skin vascular permeability that could explain human flush. Methylnicotinate induced significant Evan's blue extravasation in rat skin (Fig. 4A) that was comparable to intradermal injection of either histamine (0.01 mM) or serotonin (0.1 mM); the most potent drug was neurotensin (Fig. 4A). The niacin metabolites nicotinamide, methyl nicotinamide, nicotinamide-N-oxide and nicotinuric acid had no appreciable effect (Fig. 4B).

Effect of niacin on rat skin temperature. We also investigated the ability of niacin to induce vasodilation as determined by an increase in ear skin temperature. The normal mean ear temperature was 26.5-28.5˚C (n = 27) recorded at 9-11 AM. Niacin (10 mg/rat) was first administered either intravenously or intradermally in anesthetized rats; however, niacin failed to increase the skin temperature most likely because of interference by the anesthetics (ketamine/xylazine). Niacin (24.75 mg/kg, equivalent to 1,750 mg/80 kg human) administered ip in conscious rats induced a time-dependent temperature increase with a maximum 1.9 ± 0.2 ^{o}C (n=5, p=0.0002) that occurred at slightly different times in 4 groups of animals (30, 40, 50 and 60 min) with a mean increase at 45 min (Fig. 5A). A dose-response of niacin (8.25 – 33 mg/kg , n=5) showed maximal temperature increase of 2.0 ± 0.1 ^{o}C (p=0.001) achieved with 24.75 mg/kg rat at 45 min (Fig. 5B).

Effect of H_1-receptor antagonists ($H_1R\alpha$) on niacin-induced skin temperature increase. As histamine is a key vasodilatory molecule, we investigated whether $H_1R\alpha$ (10 min pretreatment) could prevent ip niacin (16.5 mg/kg)-induced temperature increase 45 min later.

JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Brompheniramine, chlorpheniramine, diphenhydramine and ketotifen used at their approximate maximal human equivalent doses had no effect on niacin-induced temperature increase (n=5, Fig. 6A). The heterocyclic $H_1R\alpha$ hydroxyzine, which also partially inhibits mast cell activation, inhibited the temperature increase by about 20% (n=5, $p<0.05$) and so did ($p<0.05$) the "mast cell stabilizer" cromolyn (n=5, Fig. 6A). This experiment was repeated using 5 and 10-times higher concentrations of these drugs (n=3, Fig. 6B). There was no apparent dose-response curve and the strongest inhibition was (94%) again with prochlorperazine (Fig. 6B).

Effect of serotonin receptor antagonists on niacin-induced skin temperature increase. The phenothiazine prochlorperazine, which also has serotonin receptor blocking activity, produced almost 90% inhibition of the skin temperature increase due to ip niacin (16.5 mg/kg) (n=5, $p<0.05$, Fig. 6). The mixed H_1-receptor and serotonin receptor antagonist Cyproheptadine (51.3 μg/kg, equivalent to 4 mg/80 kg) administered as one ip injection 10 min prior to ip niacin (16.5 mg/kg), completely inhibited (n=3, p=0.0036) the niacin effect measured at 45 min post-injection (Fig. 7A) were quiet effective; cyproheptadine alone actually decreased even the baseline temperature of 26.5 ^{o}C by 0.7 ± 0.2 ^{o}C (n=3, p=0.0011, Fig. 7 A).

We then investigated the inhibitory effect of different concentrations of cyproheptadine administered *6* hr prior to 16.5 mg/kg niacin administration. The inhibitory effect of cyproheptadine was evident whether its dose was 28.05 μg/kg, 42.24 μg/kg or 56.4 μg/kg (Fig. 7B). Cyproheptadine (51.3 μg/kg, equivalent to 4 mg/80 kg) reduced the effect of niacin by more than 50% (n=3, p=0.008) whether it was administered 30 min or 8 hr prior to niacin (Fig. 7C). The structurally similar drug azatadine had similar effects (not shown). These results

JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

suggested that serotonin receptor antagonism may be important. Consequently, we used the specific serotonin $5HT_{2A}$ receptor antagonist ketanserin, which inhibited niacin-induced temperature increase by 85% (n=6, p=0.0008, Fig. 8). Finally, we tested dipyridamole, which inhibits platelet aggregation; this drug inhibited niacin-induced temperature increase by 72% (n=6, p=0.0004, Fig. 8).

Effect of non-steroidal anti-inflammatory drugs (NSAIDs) on niacin-induced skin temperature increase. We also investigated whether pretreatment for 2 hr with two common NSAIDs could inhibit niacin's vasodilatory effect in this animal model (Fig. 8). ASA (1.22 mg, equivalent to 325 mg/80 kg), and indomethacin (375 µg equivalent to 100 mg/80 kg) had a weak (30%) inhibitory effect (n=6, $p<0.0001$) (Fig. 8A).

We also used 5 and 10-time higher concentrations of the serotonin receptor antagonists, and the NSAIDs (Fig. 8B). It is apparent that there is no clear dose-response, but the most potent drug were again cyproheptadine (77.8% and 94.4% inhibition) and indomethacin (44.4% and 83.3% inhibition), at their respective concentrations (Fig. 8B).

We also additionally tested all the drugs at their highest concentration used for any effect on niacin-induced serotonin release. It appears that prochlorperazine and ketanserin may also have some inhibitory effect on serotonin release (Fig. 9). In addition to their well known serotonin receptor antagonistic effect.

Discussion

Here we report for the first time to our knowledge that the niacin derivative methylnicotinate can stimulate within a few min release of significant amounts of PGD_2 from human mast cells and serotonin from human platelets. We also show that niacin increases plasma PGD_2 and serotonin in a rat model of niacin-induced flush, and that intradermal injection of niacin, methylnicotinate and serotonin induce rat skin vasodilation and vascular permeability. We finally show that anti-serotonergic drugs can inhibit niacin-induced flush in this rat model. The dose of niacin used in our study was equivalent to that previously used in the same rat model (Turenne, et al., 2001) and within the range of doses (1.5 – 2 g/day) given orally to patients with hyperlipidemia (Gupta and Ito, 2002). Drug doses 5 and 10-times higher did not produce any stronger inhibition, except in the case of cyproheptadine and indomethacin. Methylnicotinate had a weak effect on inducing histamine release from human mast cells, while niacin did not increase rat plasma histamine levels. Moreover, the H_1-receptor antagonists brompheniramine, chlorpheniramine, diphenylhydramine and ketotifen did not block niacin-induced skin temperature increases. Combined, these results suggest that histamine is not involved in niacin-induced flush.

In contrast, the phenothiazine prochlorperazine and the serotonin-receptor antagonists cyproheptadine and azatadine, as well as the specific serotonin receptor-2A antagonist ketanserin, were potent inhibitors of niacin-induced skin temperature increase in rats. The tricyclic H_1-receptor antagonist hydroxyzine, which has some inhibitory effect on mast cell activation, and the "mast cell stabilizer" cromolyn also, weakly inhibited the effect of niacin.

So far, only PGD_2 has been implicated in niacin "flush" (Morrow, et al., 1989;Morrow, et al., 1992). In one of these studies, topical superpharmacological administration of

methylnicotinate (10^{-3}-10^{-1}M) on forearm of normal volunteers dramatically increased serum PGD_2 (Morrow, et al., 1992). Niacin was shown to stimulate PGD_2 synthase in macrophages (Knowles, et al., 2005) through activation of a unique G-protein coupled receptor (Lorenzen, et al., 2002), and after 30 min stimulation resulted in a 3-fold PGD_2 release from cultured macrophages, but not from monocytes or endothelial cells (Meyers, et al., 2007). As the authors admitted, these *in vitro* findings did not reflect the amount of PGD_2 release *in vivo* or the fact that the niacin flush occurs within a few min in humans (Meyers, et al., 2007). In contrast, PGD_2 was reported *decrease* macrophage production of the potent vasodilator nitric oxide (Bellows, et al., 2005). Other reports indicated that nicotinic acid increased expression of prostanoid synthase in epidermal Langerhan's cells; depletion of these cells, but not macrophages, eliminated nicotinic acid-induced flushing (Benyo, et al., 2006). Nicotinic acid receptors have been identified (Soudijin, et al., 2007). Skin was shown to express the nicotinic acid receptor (GPR 109A) and its activation resulted in PGD_2 release; the responsible cells were again shown to be Langerhans cells (Maciejewski-Lenoir, et al., 2006). Mice lacking GPR109A did not show niacin-induced flush; nevertheless, blocking PGD_2 and PGD_2 receptors was associated with reduced, but still substantial flushing (Benyo, et al., 2005). An oral PGD_2 receptor-1 antagonist (MK-0524) could block nicotinic acid and PGD_2-induced vasodilation in female mice, but strangely only partially in male mice (Cheng, et al., 2006). Moreover, co-administration of a PGD_2 receptor antagonist with extended release niacin in humans reduced flush only by 50% (Lai, et al., 2007). These results clearly indicate that PGD_2 accounts only for part of niacin's induced flush.

Our present results further indicate that ASA has only a weak inhibitory effect on niacin-induced flush in a rat model, as also shown in clinical trials, even though it blocks serum PGD_2

levels. In one placebo-controlled study, 60% of subjects on placebo experienced "flush"-related symptoms after ingesting 500 mg immediate release niacin; there was a decrease to 41% of subjects taking daily 325 mg ASA for 4 days and to 29% subjects on 4 days of daily 650 mg ASA prior to niacin, but there was no statistical difference between the two treatments (Jungnickel, et al., 1997). In another double-blinded, cross-over study, pretreatment with 325 mg, but not 165 mg ASA or 200 mg ibuprofen, partially reduced flush-related symptoms due to 500 mg immediate release niacin (Dunn, et al., 1995). It should be pointed out that these modest (about 30%) reductions were for flush caused by 500 mg niacin, while 1500-2000 mg are required for effective treatment, suggesting that these amounts of ASA are likely to be even less effective at the higher niacin doses used clinically. A recent paper reported that a new "optimized" extended release 1000 mg niacin tablet induced flush in 89% in 156 healthy male volunteers, with a 43% reduction in median flush duration (Cefali, et al., 2006). Co-administration of ASA (650 mg) further reduced the subjects who experienced flush from 77% on placebo to 61%, while the duration of flush was reduced from 60 min to 48 min (Cefali, et al., 2007). It is obvious from the above that >60% of subjects still experience flush even on an "optimized' extended release niacin with together with ASA. Moreover, 650 mg of daily ASA could lead to gastritis.

The ability of niacin and methylnicotinate to induce serotonin release from human platelets, as well as the ability of niacin to increase rat plasma serotonin levels, and of serotonin-receptor antagonists to block niacin-induced flush in this rat model, is novel. These results could help explain the inhibitory effect of the phenothiazines haloperidol (Turenne, et al., 2001) and prochlorperazine (shown here) since they also antagonize serotonin receptors, but have no apparent effect on PGD_2 (Cosi and Koek, 2001). The vasodilatory action of serotonin on

superficial skin vasculature is apparently contrary to the *brain vessel* vasoconstrictive effects of serotonin best known from the pathophysiology of migraines. However, serotonin is the main vasodilatory amine in rodents (Askenase, et al., 1980). Moreover, serotonin is responsible for the facial flush associated with the Carcinoid syndrome in humans. In fact, during flushing of carcinoid patients, plasma serotonin levels increase significantly (Matuchansky and Launay, 1995). In addition, flushing in Carcinoid is inhibited by an antiserotonergic agent, (Loong, et al., 1968) and by cyproheptadine (Plank and Feldman, 1975). Interestingly, one paper reported that prostaglandins may control plasma serotonin levels (Utsunomiya, et al., 1981), while another reported that subthreshold serotonin concentrations potentiated the effect of low arachidonic acid on human platelet aggregation (Saeed, et al., 2003). The possibility still remains that serotonin may be inducing PGD_2 release or vice versa. At present, it is not known if PGD_2 and serotonin operate individually, sequentially, together, or indirectly by promoting the release of yet another vasodilatory molecule, such as nitric oxide.

Increasing evidence indicates the superior ability of a niacin-statin combination to reduce triglycerides, LDL, VLDL and Apo (a), while increasing HDL (Gupta and Ito, 2002;Morgan, et al., 1998;Carlson, 2005). However, the rate of niacin discontinuation due to flushing has been reported to be considerable, even with extended release niacin (Guyton, et al., 1998;McCormack and Keating, 2005) and it increases over the course of treatment (McKenney, 2004); this is also true for the combination of lovastatin and extended-release niacin (Gupta and Ito, 2002). Consequently, inhibition of niacin-induced flush is of critical importance for compliance, a problem that was recently termed the “Sixth Vital Sign” (Rosenow, 2005).

The present findings suggest that both PGD_2 and serotonin may be involved in niacin-induced flush. Plasma PGD_2 and serotonin, along with urine serotonin metabolites, should be measured in subjects receiving therapeutic amounts of niacin because no animal or *in vitro* model accurately reflects the human condition. The possibility of administering or formulating niacin together with cyproheptadine or the flavonoid luteolin, which was recently shown to inhibit niacin flush in rats (Papaliodis, et al., 2008), certainly warrants further investigation.

Acknowledgments

Thanks are due to Ms. Jessica Christian for her patience and word processing skills. We thank Amgen (Thousand Oaks, CA, U.S.A) for their generous gift of human recombinant Stem Cell Factor (hrSCF). We also thank the doctors and nurses of the department of Obstetrics and Gynecology, Tufts Medical Center, Boston, MA for collecting and providing umbilical cord blood.

References

Askenase PW, Bursztajin S, Gershon MD and Gershon RK (1980) T-cell-dependent mast cell degranulation and release of serotonin in murine delayed-type hypersensitivity. *J Exp Med* **152**:1358-1374.

Bellows CF, Alder A, Wludyka P and Jaffe BM (2005) Modulation of macrophage nitric oxide production by prostaglandin D(2). *J Surg Res* **132**:92-97.

Benyo Z, Gille A, Bennett CL, Clausen BE and Offermanns S (2006) Nicotinic acid-induced flushings is mediated by activation of epidermal langerhans cells. *Mol Pharmacol* **70**:1844-1899.

Benyo Z, Gille A, Kero J, Csiky M, Suchankova MC, Nusing RM, Moers A, Pfeffer K and Offermanns S (2005) GPR109A (PUMA-G/HM74A) mediates nicotinic acid-induced flushing. *J Clin Invest* **115**:3634-3640.

Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Bolson EL, Alaupovic P, Frohlich J and Albers JJ (2001) Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* **345**:1583-1592.

Carlson LA (2005) Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review. *J Intern Med* **258**:94-114.

Cefali EA, Simmons PD, Stanek EJ, McGovern ME and Kissling CJ (2007) Aspirin reduces cutaneous flushing after administration of an optimized extended-release niacin formulation. *Int J Clin Pharmacol Ther* **45**:78-88.

Cefali EA, Simmons PD, Stanek EJ and Shamp TR (2006) Improved control of niacin-induced flushing using an optimized once-daily, extended niacin formulation. *Int J Clin Pharmacol Ther* **44**:633-640.

Cheng K, Wu TJ, Wu KK, Sturino C, Metters K, Gottesdiener K, Wright SD, Wang Z, O'neill G, Lai E and Waters MG (2006) Antagonism of the prostaglandin D2 receptor 1 suppresses nicotinic acid-induced vasodilation in mice and humans. *Proc Natl Acad Sci U S A* **103**:6682-6687.

Cosi C and Koek W (2001) Agonist, antagonist, and inverse agonist properties of antipsychotics at human recombinant 5-HT(1A) receptors expressed in HeLa cells. *Eur J Pharmacol* **433**:55-62.

D'Souza Y, Fombonne E and Ward BJ (2006) No evidence of persisting measles virus in peripheral blood mononuclear cells from children with autism spectrum disorder. *Pediatrics* **118**:1664-1675.

Dunn RT, Ford MA, Rindone JP and Kwiecinski FA (1995) Low-dose aspirin and ibuprofen reduce the cutaneous reactions following niacin administration. *Am J Ther* **2**:478-480.

Gupta EK and Ito MK (2002) Lovastatin and extended-release niacin combination product: the first drug combination for the management of hyperlipidemia. *Heart Dis* **4**:124-137.

Guyton JR, Goldberg AC, Kreisberg RA, Sprecher DL, Superko HR and O'Connor CM (1998) Effectiveness of once-nightly dosing of extended-release niacin alone and in combination for hypercholesterolemia. *Am J Cardiol* **82**:737-743.

Jungnickel PW, Maloley PA, Vander Tuin EL, Peddicord TE and Campbell JR (1997) Effect of two aspirin pretreatment regimens on niacin-induced cutaneous reactions. *J Gen Intern Med* **12**:591-596.

Kempuraj D, Madhappan B, Christodoulou S, Boucher W, Cao J, Papadopoulou N, Cetrulo CL and Theoharides TC (2005) Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. *Br J Pharmacol* **145**:934-944.

Kempuraj D, Saito H, Kaneko A, Fukagawa K, Nakayama M, Toru H, Tomikawa M, Tachimoto H, Ebisawa M, Akasawa A, Miyagi T, Kimura H, Nakajima T, Tsuji K and Nakahata T (1999) Characterization of mast cell-committed progenitors present in human umbilical cord blood. *Blood* **93**:3338-3346.

Knowles HJ, Poole RT, Workman P and Harris AL (2005) Niacin induces PPARgamma expression and transcriptional activation in macrophages via HM74 and HM74a-mediated induction of prostaglandin synthesis pathways. *Biochem Pharmacol* **71**:646-656.

Kushnir-Sukhov NM, Brown JM, Wu Y, Kirshenbaum A and Metcalfe DD (2006) Human mast cells are capable of serotonin synthesis and release. *J Allergy Clin Immunol* **119**:498-499.

Lai E, De Leipeleire I, Crumley TM, Liu F, Wenning LA, Michiels N, Vets E, O'neill G, Wagner JA and Gottesdiener K (2007) Suppression of niacin-induced vasodialation with an antagonist to prostaglandin D(2) receptor subtype 1. *Clin Pharmacol Ther* **81**:849-857.

Libby P (2005) The forgotten majority: unfinished business in cardiovascular risk reduction. *J Am Coll Cardiol* **46**:1225-1228.

Loong SC, Lance JW and Rawle KC (1968) The control of flushing and diarrhoea in carcinoid syndrome by an antiserotonin agent, BC105. *Med J Aust* **2**:845-848.

Lorenzen A, Stannek C, Burmeister A, Kalvinsh I and Schwabe U (2002) G protein-coupled receptor for nicotinic acid in mouse macrophages. *Biochem Pharmacol* **64**:645-648.

Maciejewski-Lenoir D, Richman JG, Hakak Y, Gaidatov I, Behan DP and Connolly DT (2006) Langerhans cells release prostaglandin D2 in response to nicotinic acid. *J Inv Dermatol* **126**:2637-2646.

Matuchansky C and Launay JM (1995) Serotonin, catecholamines, and spontaneous midgut carcinoid flush: plasma studies from flushing and nonflushing sites. *Gastroenterology* **108**:743-751.

McCormack PL and Keating GM (2005) Prolonged-release nicotinic acid: a review of its use in the treatment of dyslipidaemia. *Drugs* **65**:2719-2740.

McKenney J (2004) New perspectives on the use of niacin in the treatment of lipid disorders. *Arch Intern Med* **164**:697-705.

Meyers CD, Liu P, Kamanna VS and Kashyap ML (2007) Nicotinic acid induces secretion of prostaglandin D(2) in human macrophages: An in vitro model of the niacin flush. *Atherosclerosis* **192**:253-258.

Morgan JM, Capuzzi DM and Guyton JR (1998) A new extended-release niacin (Niaspan): efficacy, tolerability, and safety in hypercholesterolemic patients. *Am J Cardiol* **82**:29U-34U.

Morrow JD, Awad JA, Oates JA and Roberts LJ (1992) Identification of skin as a major site of prostaglandin D2 release following oral administration of niacin in humans. *J Invest Dermatol* **98**:812-815.

Morrow JD, Parsons WG and Roberts LJ (1989) Release of markedly increased quantities of prostaglandin D2 in vivo in humans following administration of nicotinic acid. *Prostaglandins* **38**:263-274.

Papaliodis D, Boucher W, Kempuraj D and Theroharides TC (2008) The flavonoid luteolin inhibits niacin-induced flush. *Brit J Pharmacol* **153**:1382-1387.

Plank J and Feldman JM (1975) Adrenal function in the carcinoid syndrome: effects of the serotonin antagonist cyproheptadine. *Metabolism* **24**:1035-1046.

Rosenow EC, III (2005) Patients' understanding of and compliance with medications: the sixth vital sign? *Mayo Clin Proc* **80**:983-987.

Saeed SA, Rasheed H and Gilani AU (2003) Synergism interaction between arachidonic acid by 5-hydroxytryptamine in human platelet aggregation is mediated through multiple signalling pathways. *Acta Pharmacol Sin* **24**:958-964.

Soudijin W, van Wijngssrden I and Ijzerman AP (2007) Nicotinic acid receptor subtypes and their ligands. *Med Res Rev* **27**:417-433.

Theoharides TC, Singh LK, Boucher W, Pang X, Letourneau R, Webster E and Chrousos G (1998) Corticotropin-releasing hormone induces skin mast cell degranulation and increased

vascular permeability, a possible explanation for its pro-inflammatory effects. *Endocrinology* **139**:403-413.

Turenne SD, Seeman M and Ross BM (2001) An animal model of nicotinic-acid-induced vasodilation: effect of haloperidol, caffeine and nicotine upon nicotinic acid response. *Schizophrenia Res* **50**:191-197.

Urade Y, urihara M, Hariguchi Y, Ikai K and Hayaishi O (1989) The major source of endogenous prostaglandin D_2 production is likely antigen-presenting cells: localization of glutathione-requiring PGD2 synthetase in histiocytes, dendritic and Kupffer cells in various rat tissues . *J Immunol* **143**:2982-2987.

Utsunomiya T, Krausz MM, Shepro D and Hechtman HB (1981) Prostaglandin control of plasma and platelet 5-hydroxytryptamine in normal and embolized animals. *Am J Physiol* **241**:H766-H771.

Footnotes

Disclosure

US Patents No. 7,115,278 11/99,991, and 12/151,268 as well as EPO No. 1365777 awarded to (TCT) cover methods and composition claims for blocking niacin-induced flush.

This work was supported in part by Kos Pharmaceuticals, Inc. (Cranbury, NJ) and Theta Biomedical Consulting and Development Co., Inc. (Brookline, MA).

Legends for Figures

Figure 1. Effect of methylnicotinate (0.1 – 10 mM) on human mast cell secretion of (A) histamine and (B) PGD_2 (n=3; $p<0.05$). Histamine was assayed fluorometrically and the PGD_2 was assayed using an EIA kit.

Figure 2. A scattergram showing the effect of methylnicotinate on human platelet secretion of serotonin (n=3). Platelets were incubated in a 37°C shaking waterbath. Serotonin levels in the supernatant were assayed using an EIA kit.

Figure 3. (A) The effect of niacin and ASA on rat plasma PGD_2 levels. ASA (4.02 mg/kg) was administered for 2 hr prior to a single ip injection of niacin (24.75 ng/kg) and plasma PGD_2 levels were measured 45 min later (n=3) Brackets indicate groups compared (* p=0.018; **0.014). (B) Effect of niacin, ASA and dipyridamole on rat plasma serotonin levels. ASA (4.03 mg/kg or 402.6 µg/kg), cyproheptadine (56.43 µg/kg) and dipyridamole (4.95 mg/kg) were administered 2 hr prior to a single ip injection of niacin (11.55 mg/kg) and plasma serotonin levels were measured 45 min later (n=3, p=0.70).

Figure 4. Representative photographs of rat skin showing vasodilation and vascular permeability as shown by extravasation in response to: (A) niacin and methyinicotinate (0.01 mM) as compared to neurotensin (0.1 µM), histamine and serotonin (0.1 mM): (B) Methylnicotinate, methyl nicotinamide, niacin, nicotinamide, nicotinamide-N-oxide, nicotinuric acid (0.01 mM) neurotensin (0.1 µM), as well as serotonin and histamine (0.1 mM), and saline.

Figure 5. (A) Time-course of the effect of a single ip niacin (724.75 mg/kg) on net ear temperature increase (n=5). All time points were significant (p=0.0002). (B) Dose-response of the effect of a single ip niacin injection on net ear temperature increase recorded 45 min later (n=5). Niacin rat doses were based on 80 kg human (h) doses as follows: 8.25 mg/kg = 583 mg/human; 16.5 mg/kg = 1,167 mg/human; 24.75 mg/kg = 1,750 mg/human; 33 mg/kg = 2,334 mg/human; the effect of all doses was significant (p=0.0001).

Figure 6. (A) Effect of pretreatment (10 min) with a single ip injection of various drugs on the ear temperature increase recorded 45 min after a single ip niacin (16.5 mg/kg) injection (n=5). The rat and human equivalent doses were: brompheniramine (56.43 μg/kg = 4 mg/human); chlorpheniramine (56.43 μg/kg = 4 mg/human); diphenhydramine (706.2 μg/kg = 50 mg/human); cromolyn (282.81 μg/kg = 20 mg/human); hydroxyzine (706.2 μg/kg = 50 mg/human); ketotifen (56.43 μg/kg = 4 mg/human); prochlorperazine (141.24 μg/kg = 10 mg/human). The effect of these drugs was compared to that of niacin ($^{*}p<0.01$). (B) This experiment was repeated using 5 and 10-times higher concentrations of these drugs as shown (n=3).

Figure 7. (A) Inhibitory effect of a single ip injection of cyproheptadine (51.3 μg/kg rat= 4 mg/h) administered 10 min prior to ip niacin (N) (7.5 mg/rat) on the ear temperature recorded 45 min later (n=3); cyproheptadine (C) alone reduced the basal skin temperature and inhibited the increase induced by niacin (p=0.001). (B) Dose-response of the inhibitory effect of cyproheptadine (C), administered ip 6 hr prior to a single niacin (N) injection (16.5 mg/kg) on the ear temperature increase recorded 45 min after niacin (n=3; p=0.005). (C) Effect of

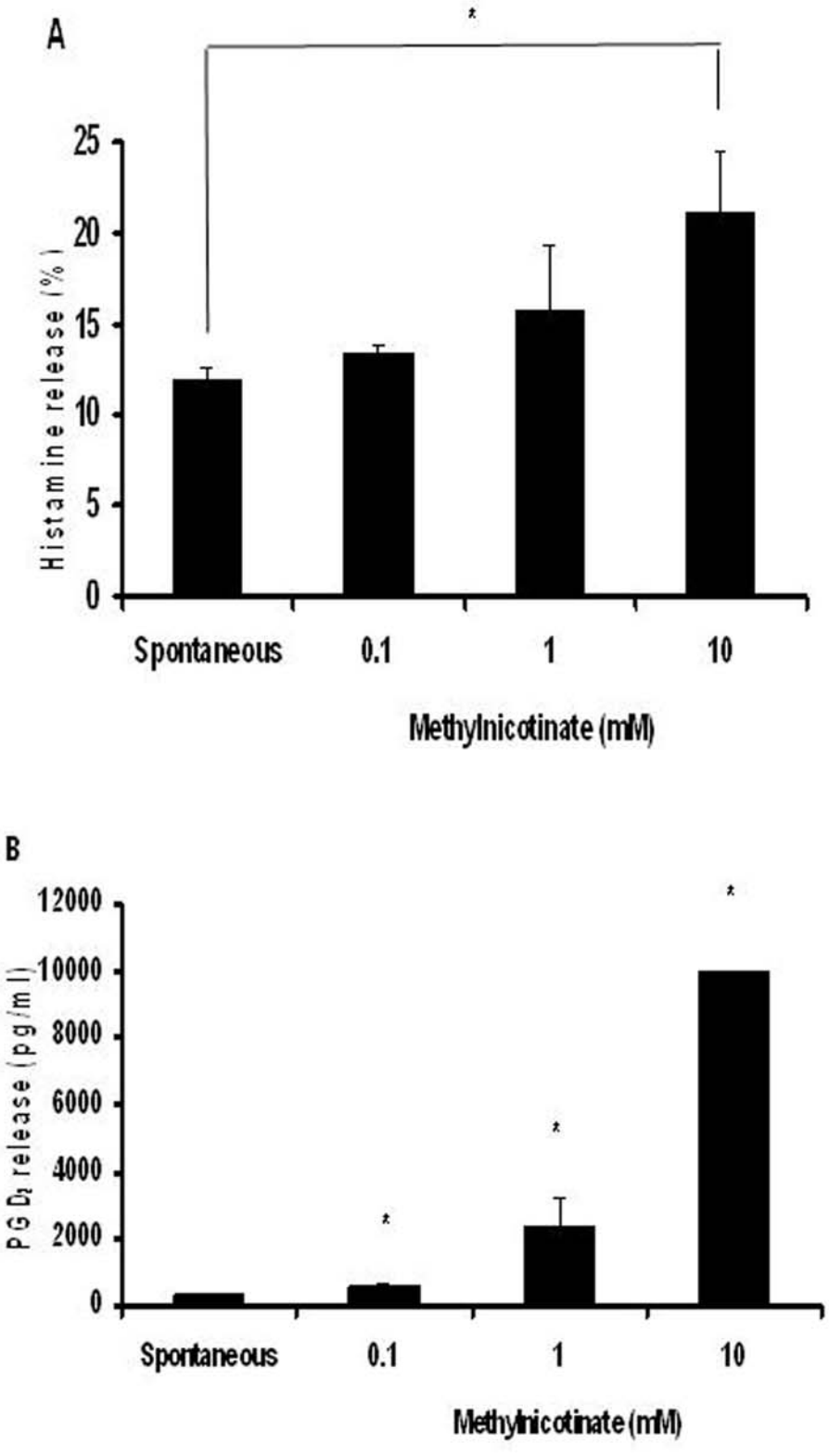
pretreatment with a single ip injection of cyproheptadine (51.3 μg/kg = 4 mg/h) at various times on the ear temperature increase recorded 45 min after a single ip niacin (16.5 mg/kg) injection (n=3; p=0.008).

Figure 8. (A) Comparison of the inhibitory effect of ASA (4.026 mg/kg = 325 mg/human), cyproheptadine (28.05 μg/kg = 1.6 mg/human), dipyridamole, (4.95 mg/kg = or 400 mg/human), indomethacin (1237.5 μg/kg = 100 mg/human), and ketanserin (660 μg/kg = 40 mg/human) administered ip 10 min prior to niacin on the ear temperature increase recorded 45 min after a single ip niacin (24.75 mg/kg) (n=6,*p=0.0008). (B) Experiments with 5 and 10-time higher concentrations of the serotonin receptor antagonists as shown (n=3).

Figure 9. Effect of the highest concentration of inhibitors on niacin-induced rat plasma serotonin levels. The rats (n=3) were injected with or without inhibitors at the concentrations shown 10 min before niacin administration. Plasma was collected and serotonin assayed using an EIA kit.

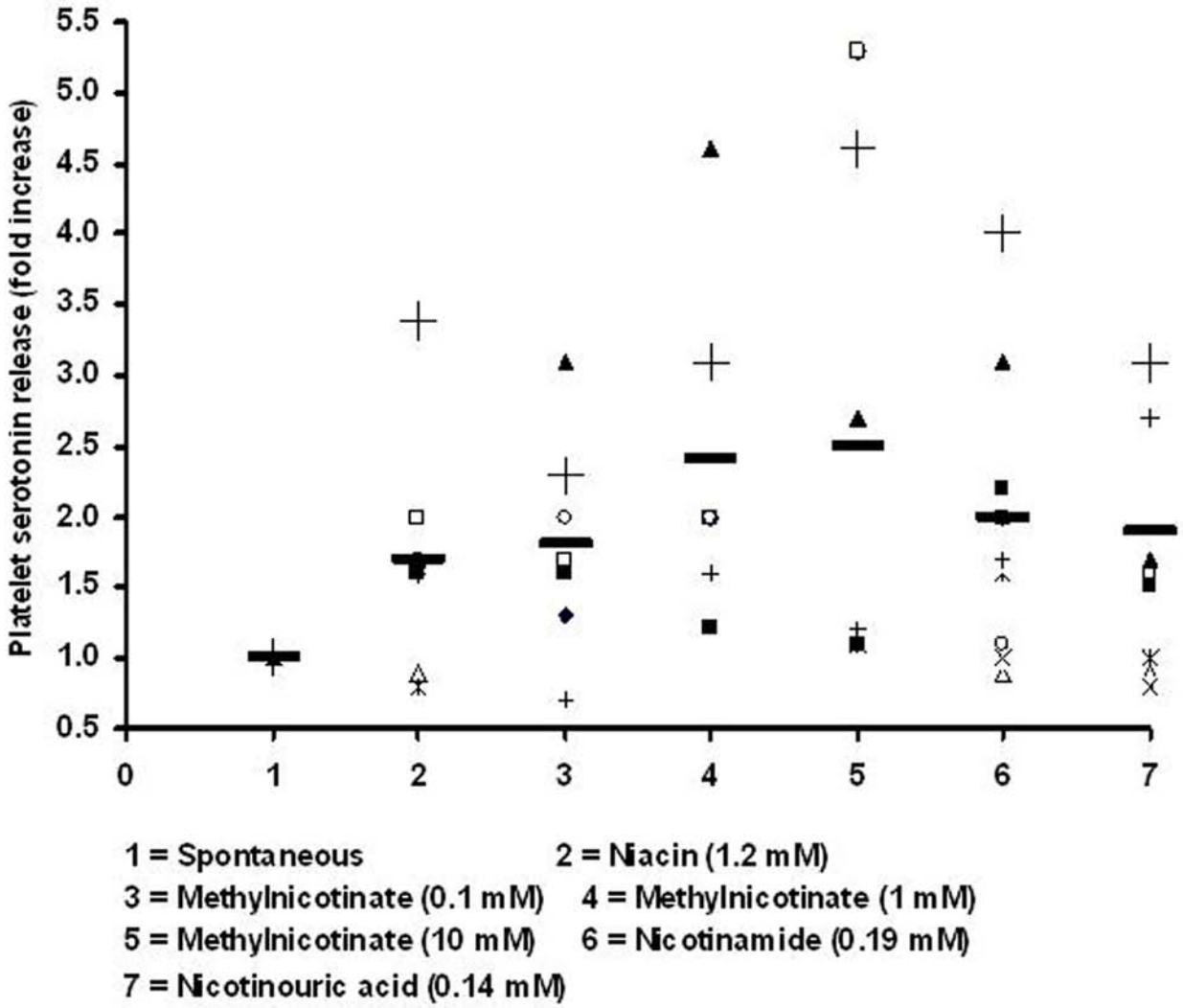
JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Fig.1.



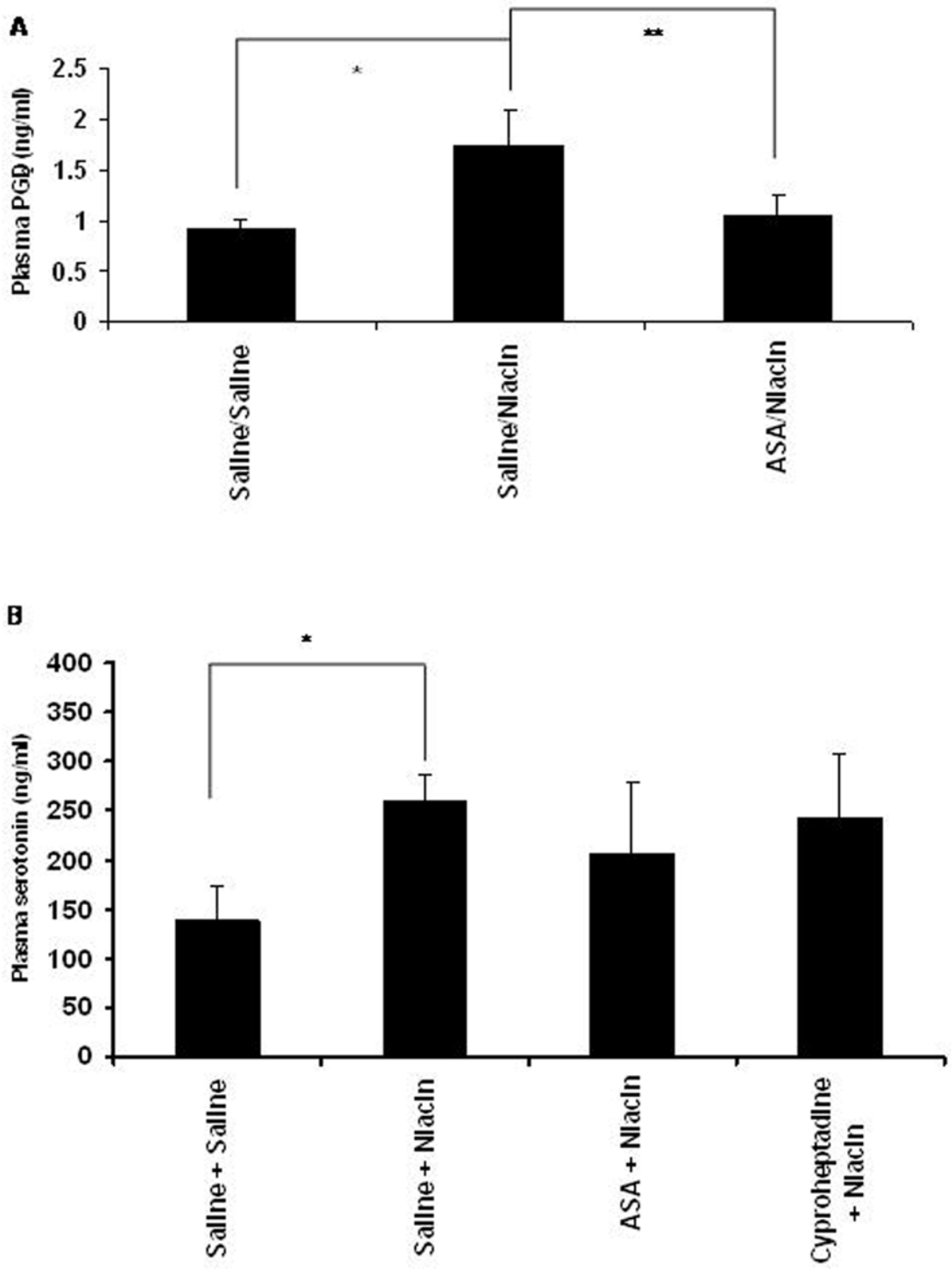
JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Fig.2.



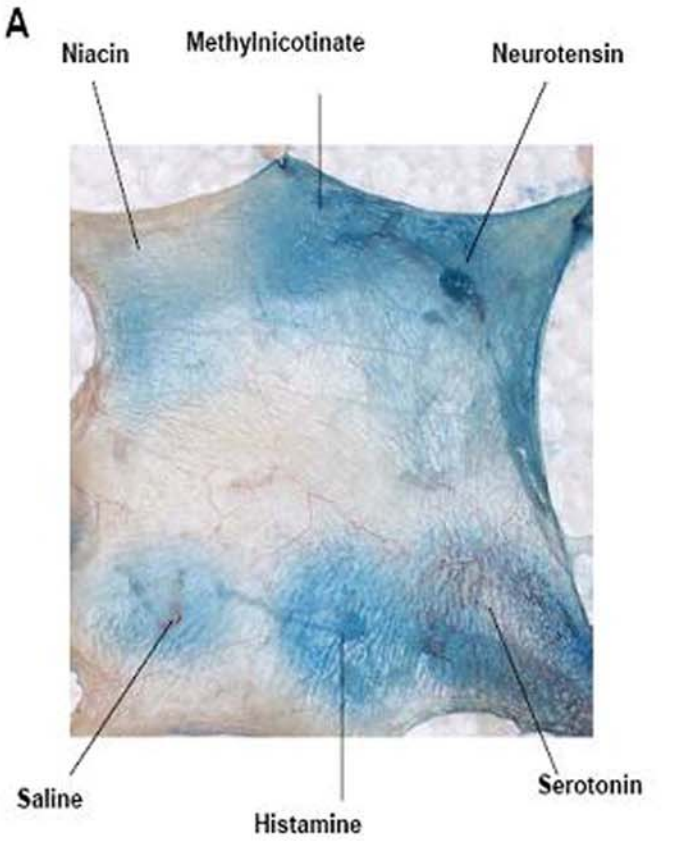
JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Fig.3.



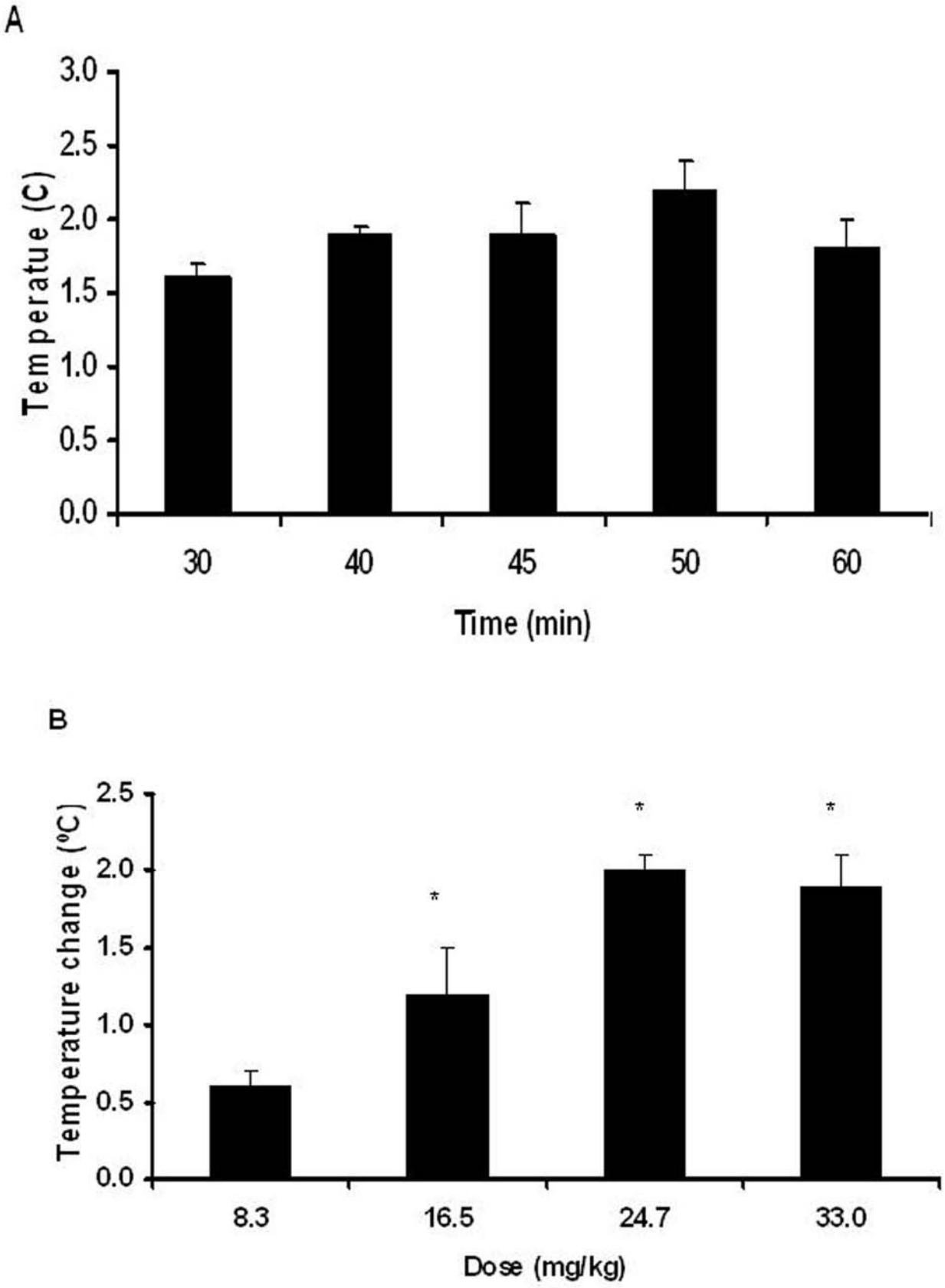
JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Fig.4.



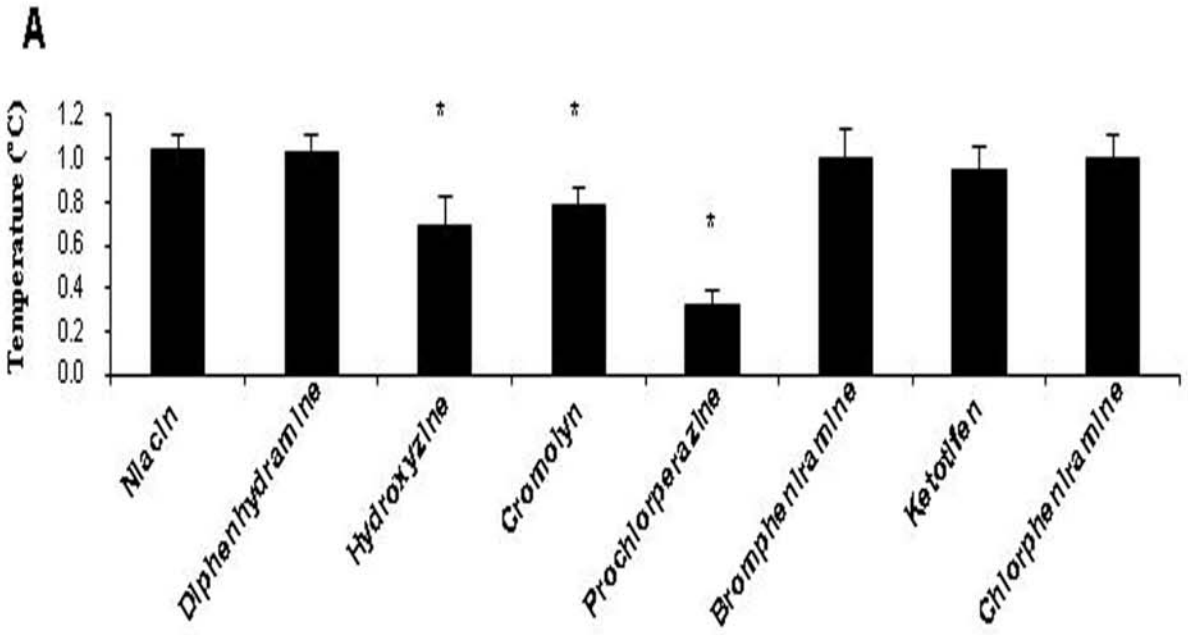
JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Fig.5.



JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 6



JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Figure. 6

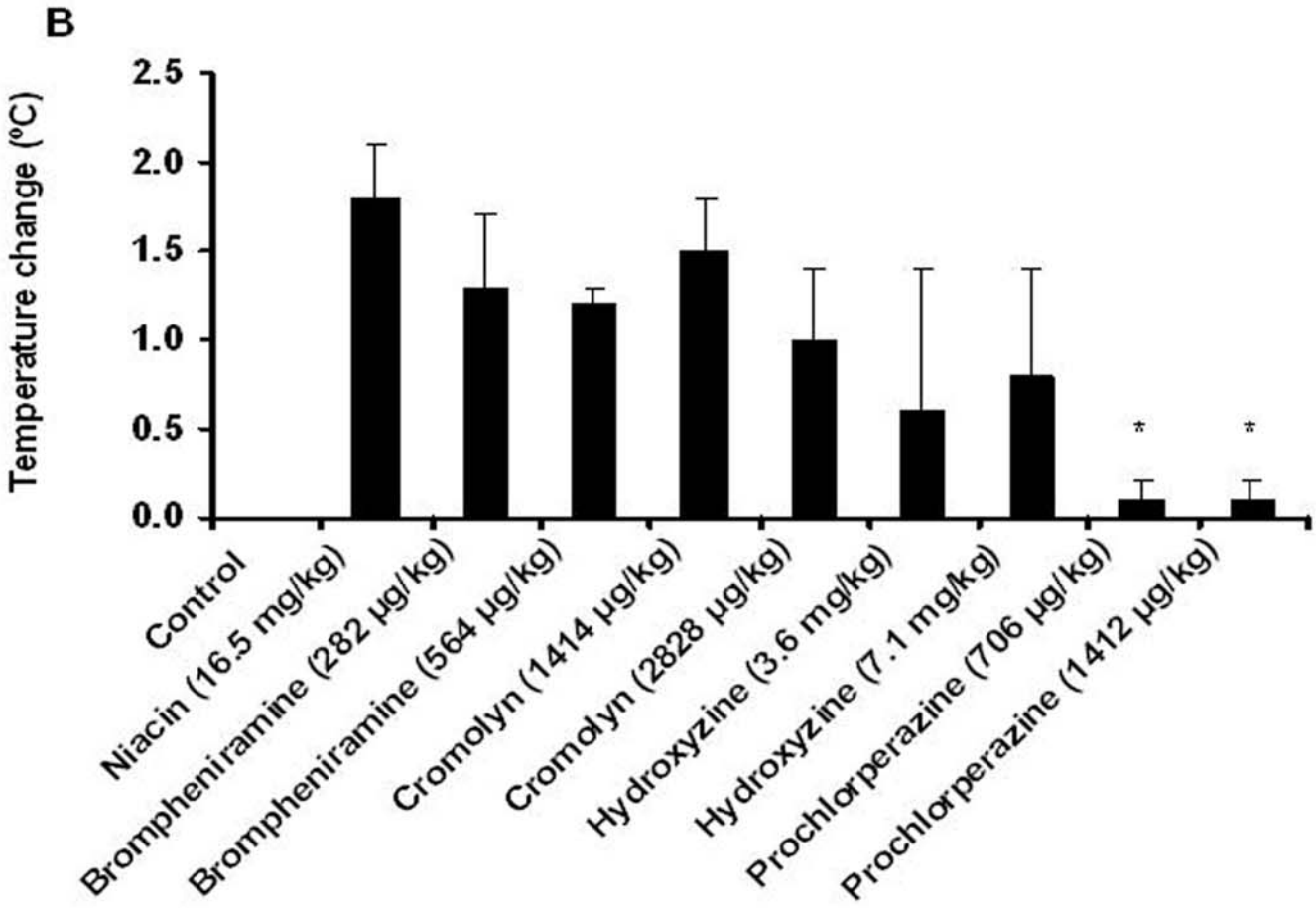
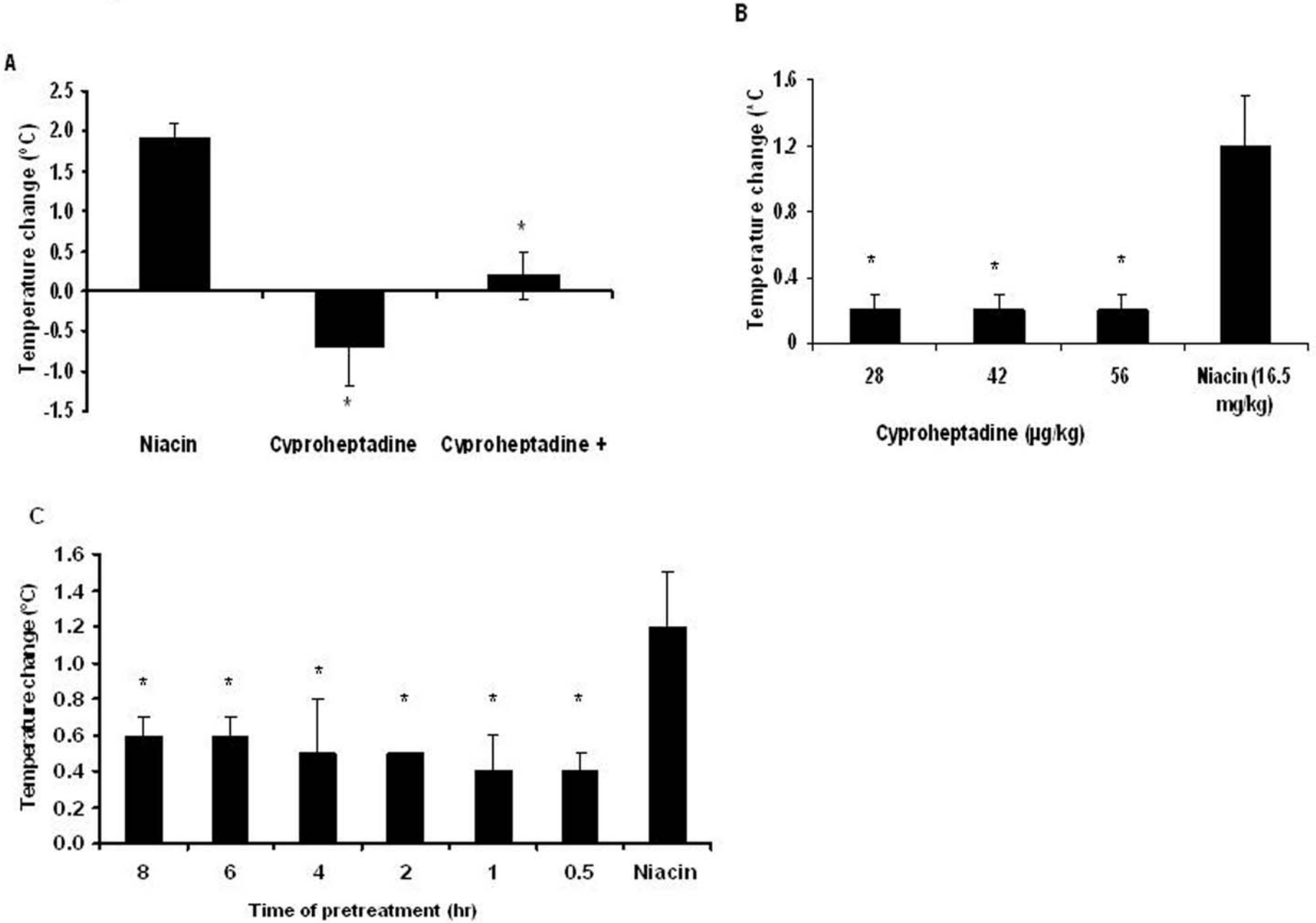
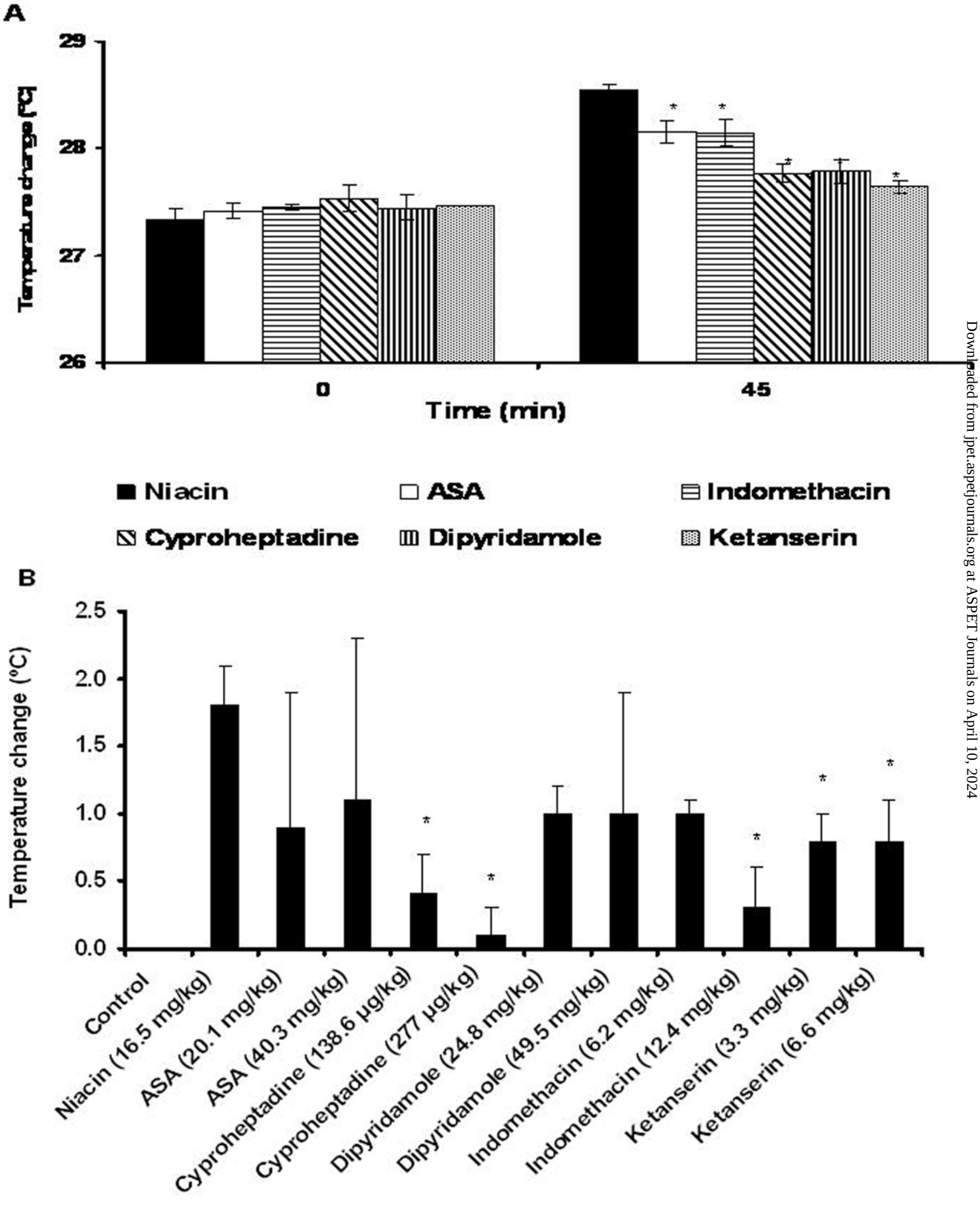


Fig.7.



JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

Fig.8.



JPET Fast Forward. Published on September 10, 2008 as DOI: 10.1124/jpet.108.141333
This article has not been copyedited and formatted. The final version may differ from this version.

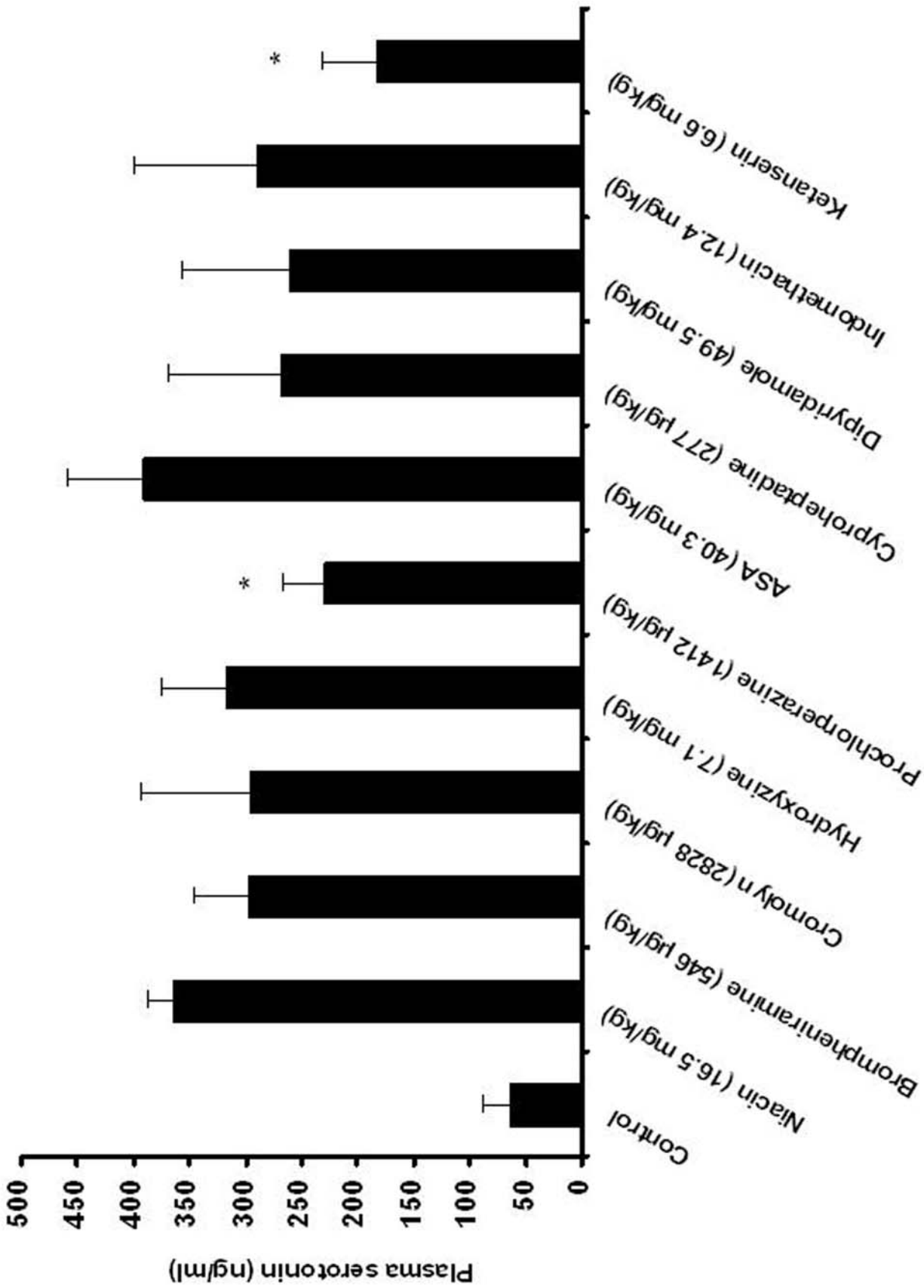


Fig.9.