

**Effect of Bupropion on hippocampal dopamine, serotonin and noradrenaline
and on peripheral hormonal concentrations in the rat: an in vivo study**

Running title: central and peripheral effects of Bupropion

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Abstract

The purpose of the present study was to administer an acute dose of the dual dopamine noradrenaline reuptake blocker bupropion in freely moving rats and to monitor the extracellular neurotransmitter concentrations in the hippocampus via in vivo microdialysis and the peripheral hormonal concentrations via catheterization. A microdialysis probe was inserted in the hippocampus and samples for serotonin, dopamine and noradrenaline were collected every 20 minutes before and after the injection of 17 mg kg^{-1} of bupropion for a total sampling time of 180 minutes. A catheter was placed in the vena femoralis of the second group of rats and blood samples were collected before and after bupropion injection for quantification of growth hormone, prolactin (PRL), corticosterone, adrenocorticotropin hormone and beta-endorphins. All neurotransmitter levels (dopamine, noradrenaline and serotonin) significantly increased after bupropion injection. This was accompanied by a significant decrease in PRL concentrations while the other hormones showed no statistically significant variation. It can therefore be concluded that, although bupropion has dual reuptake properties, the observed effects both at the central and at the peripheral level seem to be ruled by the dopaminergic system.

Keywords: dopamine/noradrenaline reuptake inhibitor, hippocampus, hypothalamic pituitary adrenal axis, microdialysis, neurotransmitters.

Introduction

The role of neurotransmitter release in the regulation of the hormonal response to exercise has drawn the attention of researchers because of a possible influence on the outcome of performance and fatigue. In our previous research we focused on these integrative mechanisms, using athletes and exercise training as methods to evaluate acute and chronic (physical) stress (Meeusen et al. 1997, 2001, Piacentini et al. 2002 a & b). In our latest study we tested the effects of a clinically used dose of bupropion on performance (Piacentini et al. accepted for publication), using the neuroendocrine response to exercise to hypothesize the role of the central neurotransmitter systems.

Catecholamines in general are known to play a role in arousal, mood, motivation, vigilance, anxiety and reward mechanisms, and could therefore, if adversely affected, impair performance (Davis, 1997). Bupropion is a dual dopaminergic/noradrenergic reuptake inhibitor, presenting weak but relatively selective inhibition characteristics of dopamine reuptake. Its potency as an inhibitor of noradrenaline reuptake is half of that of for dopamine and it shows little affinity for the serotonergic transport system (Ascher et al. 1995). Dopamine is known to have an effect on the release of certain pituitary hormones (Gilbert 1995), in particular on PRL. Laakman and colleagues (1982) however, failed to find an effect of bupropion on PRL and GH in humans. Therefore the purpose of the present study was to evaluate the effects of an acute dose of bupropion on the 5-HT, DA and NA extracellular hippocampal concentrations and on the peripheral hormonal concentrations, in order to understand the direct effect of this manipulation on brain neurotransmitters and on the hypothalamic pituitary adrenal axis (HPA).

Methods

Animals

Male albino Wistar rats (260-320 g) were used in all experiments. Animals were housed in cages (3 per cage) with food and water available ad libitum. 6 rats were used for the microdialysis experiments and 6 for the catheterization.

The procedures used in this study were carried out according to the European Guidelines on Animal experimentation and were approved by the ethical committee of the Faculty of Medicine, of the Vrije Universiteit Brussel

Microdialysis experiments

Surgery and intrahippocampal dialysis

Animals were anesthetized with an intraperitoneal injection of a mixture of diazepam (5 mg kg⁻¹) and ketamine HCl (50 mg kg⁻¹) and were placed on a stereotaxic frame.

A cannula with a replaceable guide (CMA Microdialysis, Stockholm, Sweden) was implanted through a burr hole in the hippocampus (x: -4.6, y: -5.6, z: +4.6) according to the coordinates described by Paxinos & Watson (1986). The cannula was secured to the skull using dental cement (Durelon Germany) Post-operative analgesia was provided to each rat by giving a single injection of ketofen (4 mg kg⁻¹ i.p)

Once in the cage, a microdialysis probe with a membrane length of 3 mm (CMA Microdialysis, Stockholm, Sweden) was inserted. The microdialysis probe was connected to a CMA 100 microdialysis pump (CMA microdialysis, Stockholm, Sweden) and was perfused with a modified Ringer's solution (147 mmol L⁻¹ NaCl, 4 mmol L⁻¹ KCl, 2.2 mmol L⁻¹ CaCl₂) at a flow rate of 2µL.min⁻¹. Dialysate sampling was started after a minimal period of 12 h following the

completion of surgery, permitting the animals to recover sufficiently. During the experiment, dialysates were collected every 20 min from the freely moving animals.

Experimental procedures

After 2 h of baseline collections, animals received an i.p. injection of 17 mg kg⁻¹ of bupropion (GlaxoSmithKline Hertfordshire, United Kingdom). This particular dosage was chosen because from previous literature (Nomikos et al. 1989 Terry et al. 1997) it seems to be a relatively low dose (not in the lowest range) however sufficient to increase central neurotransmitter release. Sampling continued for 140 min (7 collection samples). Samples were collected and analysed for 5-HT, DA and NA. 17 mg kg⁻¹ of bupropion were considered to be a dose that would have an effect on neurotransmitters and could be comparable to the dose we gave in humans that was the highest possible without having any side effects. Bupropion was kindly supplied by GlaxoSmithKline (Hertfordshire, United Kingdom).

Chromatographic Assays for the determination of noradrenaline, dopamine and serotonin in hippocampal microdialysates.

For the analysis of dopamine, serotonin and noradrenaline, an off-line microbore liquid chromatography assay (C8, 5 µm; 100 x 1 mm) was used with automatic injection (10 µl) of the samples, as described previously in detail (Sarre et al., 1997). In summary, the assay was based on ion-pair reversed phase chromatography, coupled to single-channel electrochemical detection (Decade, Antec, Leiden, The Netherlands). The mobile phase for serotonin and dopamine consisted of 28 ml acetonitrile and 200 ml of the following aqueous buffer: sodiumacetate trihydrate (0.1 M), citric acid monohydrate (20 mM), decane sulfonic acid (2 mM) and sodium EDTA (0.5 mM) adjusted to pH 5.5 while the mobile phase for noradrenaline consisted of 21-23 ml of acetonitrile and 200 ml of the previously described buffer. The flow rate through the column

was $90 \mu\text{l}\cdot\text{min}^{-1}$. Because of the high pH 5.5 of the mobile phase, a low oxidation potential was set (+450 mV vs. Ag/AgCl). The retention times for noradrenaline, dopamine and serotonin were 3, 7 and 14 min respectively with a quantification limit for all compounds of 20-40 pM..

Catheterization experiments

Surgical intervention

Animals were anesthetized with intraperitoneal injection of a mixture of diazepam (5 mg kg^{-1}) and ketamine HCl (50 mg kg^{-1}).

First a heparin coated polyurethane catheter (Solomon Scientific, Harleysville PA USA) was tunneled subcutaneously and exited at the back of the neck. Then the catheter was implanted in the femoral vein and continuously perfused with saline (flow rate: 0.05 ml/min). The catheter was used for blood sample collection (1 ml).

Experimental procedure

After the surgery, rats were placed in the cage and the start of the experiment was about 1 hour after full awakening. A 1 ml blood sample was taken at baseline, 20 minutes after bupropion injection (17 mg kg^{-1} i.p.) and 2 more samples at 40 minute time intervals. After each sample the same volume of saline was injected. The catheter was attached to saline enriched with 1 ml of heparin (LEO Pharma N.V/S.A, Zaventem, Belgium) and rinsed the catheter with a constant flow.

Analytical procedure for the determination of the hormones in the rats plasma.

Blood samples for the quantification of adrenocorticotropin hormone (ACTH) and beta-endorphins (β -E) were collected in pre-frozen 4.5 ml K_3 EDTA vacutainer tubes (Beckton

Dickinsons Vacutainer System Europe, Belliver Industrial Estate, Plymouth, UK) immediately centrifuged at 3000 RPM (Labofuge, Heraeus Christ, Van Der Heyden, Brussels, Belgium) for 10 min and frozen at -20°C until further analysis. Blood samples for quantification of cortisosterone, growth hormone (GH) and prolactin (PRL) were collected in 8.5 ml vacutainer serum tubes (Beckton Dickinsons Vacutainer System Europe, Belliver Industrial Estate, Plymouth, UK), were kept at room temperature until clotting before centrifuging at 3000 RPM (Labofuge, Heraeus Christ, Van Der Heyden, Brussels, Belgium) for 10 min.

Samples were then assayed via radioimmuno assay (RIA) for ACTH and beta-endorphins (Phoenix Peptide Belmont, CA, U.S.A), PRL (DRG Instruments GmbH Marburg, Germany), GH (Linco Res. Inc. St. Charles, Ms, USA), cortisol (DSL Sinsheim, Germany).

Statistical Analysis

Data from the microdialysis experiments are presented as mean \pm SEM (nM). The 6 baseline collections (pre-injection collections) were averaged and all data were analysed by One-way ANOVA for repeated measures. If the overall F-test showed significance, then post-hoc analysis was performed using the Fischer PLSD-test in order to evaluate statistical significance. Significance was set at $P < 0.05$. Hormonal data are presented as mean \pm SEM. Comparisons with baseline were analysed by One-way ANOVA for repeated measures. If the overall F test showed significance, then post-hoc analysis was performed using the Fischer PLSD-test. Significance was set at $P < 0.05$.

Results

Effects of bupropion on extracellular NA, 5-HT and DA concentrations in the hippocampus of freely moving rats. (Figure 1)

Extracellular DA levels increased significantly (3.5 fold) reaching their highest value 40 minutes post injection (0.10 ± 0.01 nM to 0.37 ± 0.10 nM). These DA concentrations remained significantly elevated until $t_{60 \text{ min}}$. After that, the levels decreased but did not return to baseline values 2.5 hours post injection.

Similar to that observed for DA, extracellular NA levels increased about 3.5 fold after bupropion injection. They remained elevated until 120 minutes post-injection. The peak concentration for NA was observed after 40 minutes: they increased from 0.16 ± 0.03 nM to 0.40 ± 0.03 nM. Afterwards, the NA levels started to decrease but did not return to baseline levels by the end of the experiment.

5-HT showed no significant change after bupropion injection.

Effects of Bupropion on peripheral hormonal concentrations (Figure 2)

PRL plasma concentrations decreased and reached a 75% decrease 100 minutes post injection (from 23.5 ± 7.2 ng.ml⁻¹ to 5.7 ± 4.1 ng.ml⁻¹). All the other hormones showed no significant change after bupropion injection.

Discussion

The importance of linking central and peripheral measurements derives from the increasing interest in understanding the specific effect of neurotransmitters on the outcome of performance.

To our knowledge, this is the first study to evaluate the peripheral and central effects of bupropion in freely moving animals.

The effects of bupropion on the HPA axis has been previously studied only in humans (Laakman et al. 1982, Piacentini et al. accepted for publication) and neither found an effect on PRL and GH. The purpose of the present study was to evaluate whether bupropion, a dual dopaminergic noradrenergic reuptake inhibitor, would increase hippocampal NA and DA extracellular concentrations, and to evaluate whether the reuptake properties of the drug were associated with peripheral hormonal concentrations.

Bupropion is a weak but relatively selective inhibitor of DA reuptake. Its potency as an inhibitor of NA reuptake is half of that of DA and it shows little affinity for the serotonergic transport system (Ascher et al. 1995). The major metabolites of bupropion, (HB) and threohydrobupropion are weaker inhibitors of DA, 5-HT and NA reuptake (Ascher et al. 1995). Although bupropion is a more potent inhibitor of DA reuptake than other antidepressants, its metabolites are not (Ascher et al. 1995).

Microdialysis.

The choice of the hippocampus as area of interest relates to the fact that there is a mutual influence of the hippocampus on the output of hormones and on the effect of the same hormones on hippocampal function. The principal circulating glucocorticoid in rodent, corticosterone (cortisol in human) targets receptors throughout the body, but also in the brain and in particular the hippocampus has plenty of glucocorticoid and mineralcorticoid receptors (Lathé 2001). There is therefore evidence for a modulation of hippocampal function by hormones as well as a direct control of the hippocampus on the HPA axis and the production of downstream hormones,

including but not restricted to adrenal glucocorticoids and catecholamines (for review see Lathe 2001).

The results of the present study demonstrate that an acute dose of bupropion increases the extracellular concentrations of DA and NA in the hippocampus of freely moving rats with no effect on 5-HT. Both microdialysis and electrophysiological studies show that acute doses of bupropion have an effect on DA and NA in the striatum and nucleus accumbens in a dose dependent manner (Cooper et al. 1994, Nomikos et al. 1989). Bupropion, but not its metabolites, reduced the firing rates of dopaminergic neurons in a dose dependent manner (Ferris & Cooper 1993). It is evident from the present and the previous animal studies that bupropion is able to increase DA and NA in different brain regions.

Hormonal results

As expected, PRL concentrations decreased immediately after injection reaching a 75% decrease 100 minutes after injection. It is now well established that the release of PRL is tonically inhibited by central mechanisms in which DA is involved (Checkeley, 1980). However NA, via activation of the alpha 2 receptors has an inhibitory influence on PRL release as well (Kiem et al. 1995). This result is confirmed by a previous study that measured GH and PRL concentrations in man and in rats after an acute bupropion injection. The authors (Stern et al. 1979) found, as we did, a decrease in PRL and no effect on GH concentrations. Human studies failed to find a decrease in PRL concentrations after different doses of bupropion (Laakman et al. 1982, Piacentini et al. accepted for publication), as expected by a dopaminergic reuptake inhibitor. The pharmacological profile of bupropion however, is different in humans and in animals due to the fact that rodents lack the metabolite hydroxybupropion (HB) (Cooper et al. 1994). In humans the effects of bupropion may result from the large concentration of the metabolite, which has mainly

noradrenergic effects (Cooper et al. 1994), and this would confirm the different hormonal response between humans and animals.

DA is known to have effects on PRL and GH, but has no effects on ACTH and cortisol (Checkeley 1980) or could even be involved in mechanisms that inhibit ACTH release (Checkeley 1980). In the present study in fact ACTH and beta endorphins were not affected by bupropion most probably due to the inhibitory role of DA on the release of the pituitary hormones (Gilbert 1995).

Peripheral hormone concentrations as markers for central effects

The combination of these central and peripheral data are important for researchers interested in the role of neurotransmitters on performance, and fatigue. In particular, the hippocampus itself has an important role on stress reactivity (Lupien & Lepage 2001). If adrenal steroids typically have adaptive effects in the short run, they promote pathophysiology when there is either repeated stress or deregulation of the HPA axis and the feedback mechanisms to the hippocampus may be deregulated (McEwen 2001).

PRL has been considered the peripheral marker of serotonergic function but it might be that when the DA influence e.g. from hippocampus is present (as in this experiment) the reaction is more complex. Similarly, supplementing humans with fluoxetine, a 5-HT reuptake inhibitor, (Meeusen et al. 2001), the PRL increase due to the reuptake inhibitor during exercise was lower when compared to the placebo trial. This could again indicate that there was a dopaminergic increase secondary to the drug-induced 5-HT increase (Santiago et al. 1998) that influenced the PRL response to exercise. It seems, according to the present and past results, that PRL can be considered a better marker for dopaminergic than for serotonergic central activity.

In summary, the weak but relatively selective inhibition of DA reuptake due to bupropion is confirmed by the present microdialysis data: a significant increase in hippocampal DA and NA concentrations. The peripheral hormonal output seems to be regulated more by the dopaminergic system, due to the fact that we found a decrease in PRL and no effect on the other hormones that would have increased under the noradrenergic effect alone.

Acknowledgements:

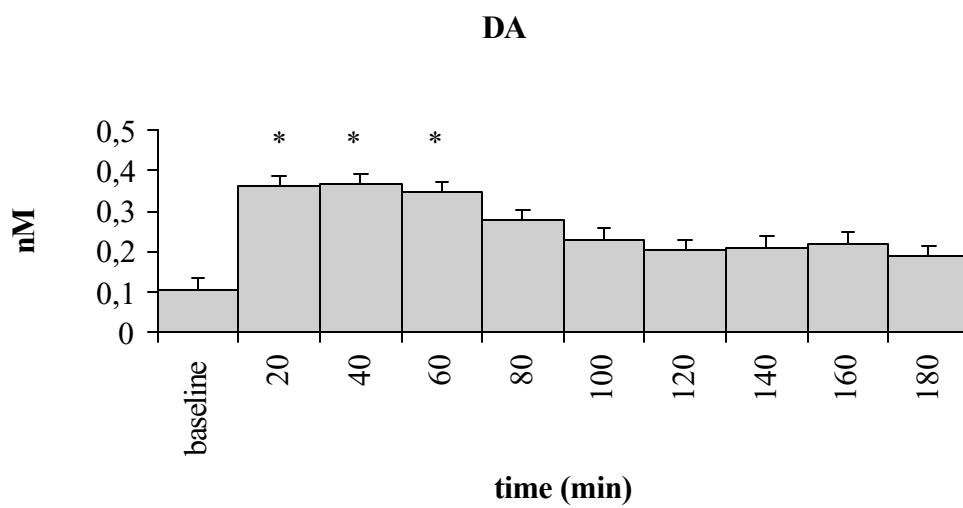
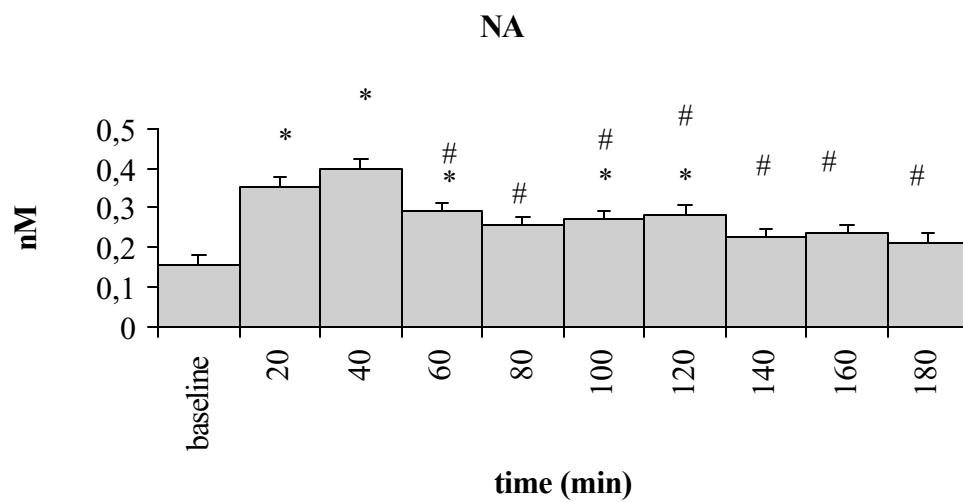
The authors appreciate the excellent technical assistance provided by Mrs. Ria Berckmans, Mr. G. De Smet, Mrs. C. De Rijck and Mrs. R. M. Greens. This research was supported by the Research Council of the Vrije Universiteit Brussel (OZR 387-607).

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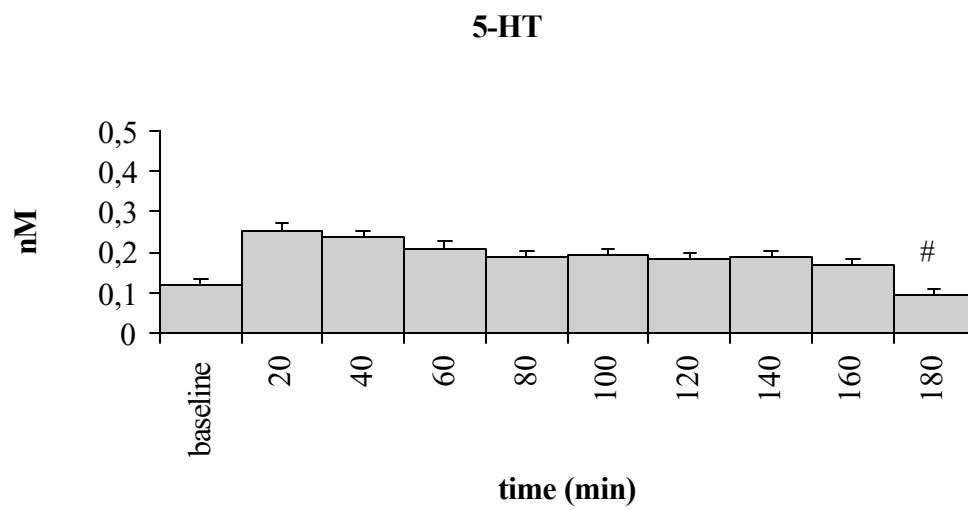
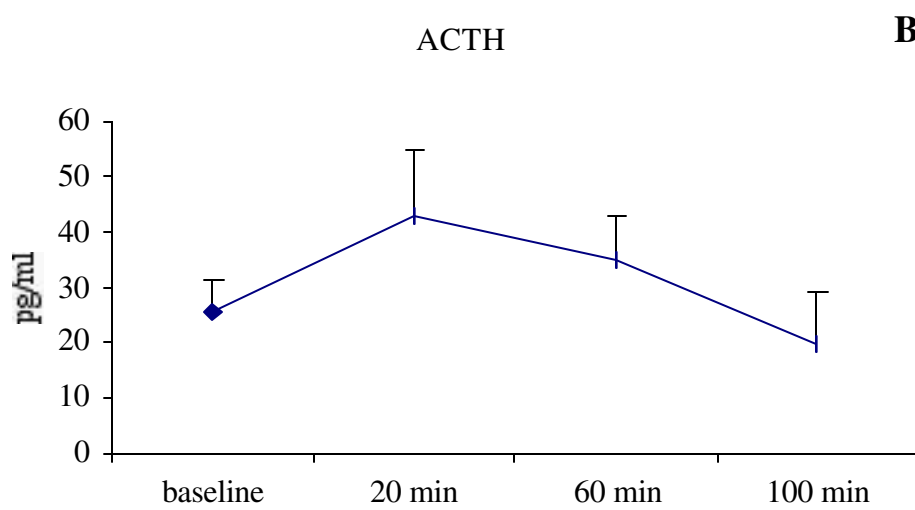
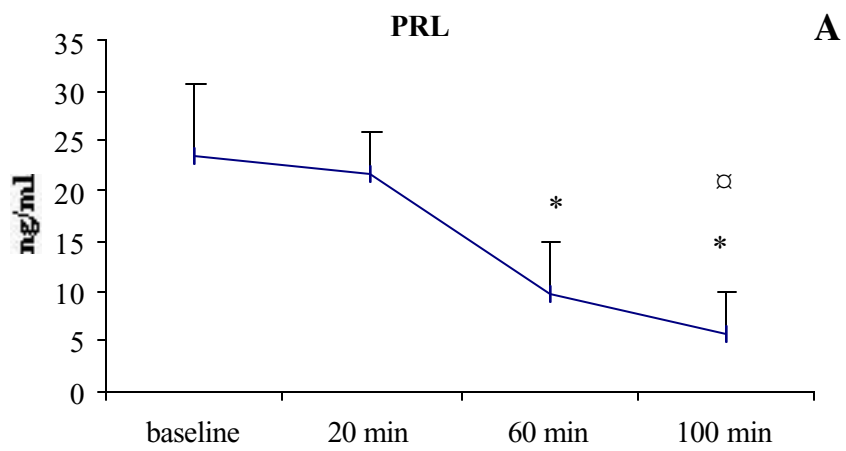


Figure 1: Effect of bupropion (17 mg/kg i.p.) on extracellular noradrenalin (NA) (**upper panel**), dopamine (DA) (**middle panel**); and serotonin (5-HT) (**lower panel**); concentrations in the hippocampus of the freely moving rat. Microdialysis samples were collected every 20 minutes. Data are presented in nM as mean \pm SEM (n=6) and were analysed by one-way ANOVA for repeated measures followed by Fischer PLSD post-hoc test ($\alpha = 0.05$)

* = statistically significantly different from baseline values

#= statistically significantly different from the 40 minute time point



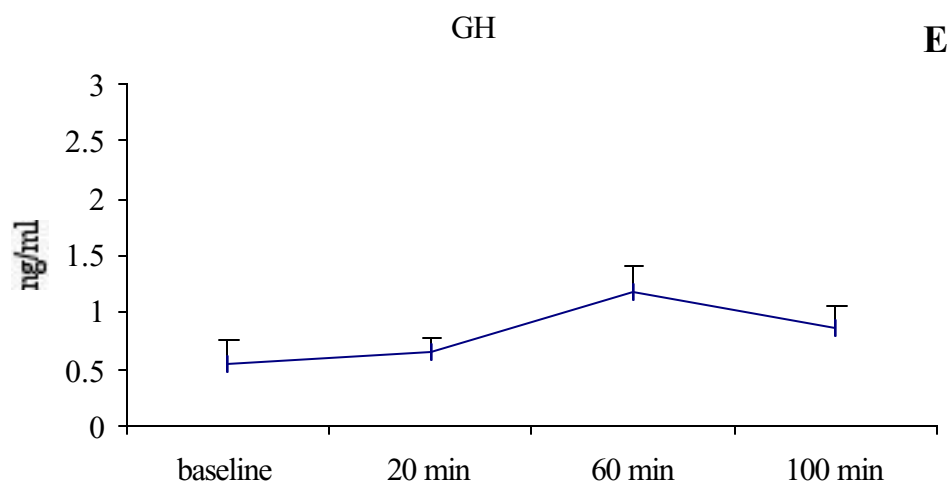
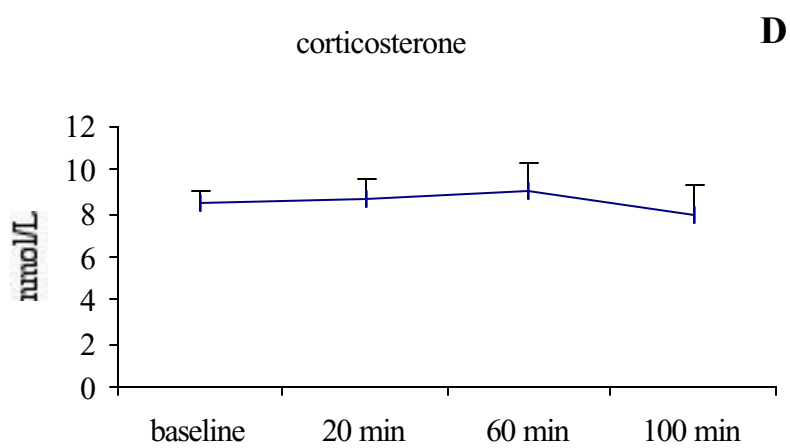
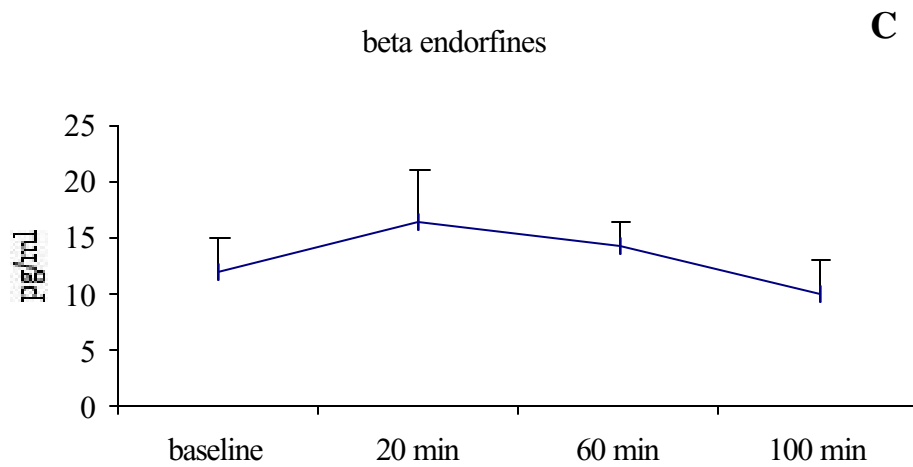


Figure 2: Effect of bupropion (17 mg/kg i.p.) on plasma concentrations of Prolactin (PRL) (**A**), ACTH (**B**), beta-endorphins (**C**) corticosterone (**D**) and GH (**E**) in freely moving rats. Data are presented as mean \pm SEM (n=6) and were analysed by one-way ANOVA for repeated measures followed by Fischer PLSD post-hoc test ($\alpha = 0.05$)

* = statistically significantly different from baseline values

§= statistically significantly different from the 20 minute time point