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SHORT COMMUNICATION

GLYCYRRHETINIC ACID, AN INHIBITOR OF 11β-HYDROXYSTEROID DEHYDROGENASE, ALTERS LOCAL CEREBRAL GLUCOSE UTILIZATION *IN VIVO*

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Summary—11β-Hydroxysteroid dehydrogenase (11β-OHSD) metabolizes corticosterone (B) to inactive 11-dehydrocorticosterone and thus protects the non-specific renal mineralocorticoid receptor from exposure to B *in vivo*. There is regional 11β-OHSD mRNA expression and bioactivity in brain *in vitro*, but any *in vivo* function is unknown. We used the [^{14}C]2-deoxyglucose technique in conscious rats to investigate whether 11β-OHSD inhibition with glycyrrhetinic acid alters local cerebral metabolic activity. We found increased glucose use in subregions of the hypothalamus, hippocampus, neocortex and subthalamus. Thus, 11β-OHSD may play a role in regulating the effects of B in the brain, *in vivo*.

INTRODUCTION

The physiological glucocorticoid corticosterone (B) and the mineralocorticoid aldosterone act on the brain to effect changes in cerebral function. These actions are mediated by binding to two types of intracellular receptor, glucocorticoid (type II, GR) and mineralocorticoid (type I, MR) [1]. However, *in vivo* binding studies have suggested that brain corticosteroid receptors are more heterogenous. Thus, most MR in the hippocampus bind both aldosterone and B, whereas apparently structurally identical MR in the anterior hypothalamus/preoptic area and periventricular regions are selective for aldosterone [2, 3]. Cerebral GR also show regional differences in B sensitivity [3], although this may be mediated via ligand binding to co-localized MR [4]. The mechanism operating to produce these site-specific differences is unknown.

Purified or expressed recombinant MR bind B and aldosterone with equal affinity *in vitro* [1, 5, 6]. *In vivo*, however, renal MR are aldosterone selective, despite a 1000-fold excess of circulating B [7]. This *in vivo* selectivity is conferred by 11β-hydroxysteroid dehydrogenase (11β-OHSD) which rapidly metabolizes B (cortisol in man) to inactive 11-dehydrocorticosterone (cortisone), thus preventing access of physiological glucocorticoids to the non-selective MR [8, 9]. Inhibition of 11β-OHSD by liquorice or its active derivative glycyrrhetinic acid (GE) allow glucocorticoids to gain access to and activate renal MR. GE itself has very weak affinity for MR and its effects are mediated by inhibition of 11β-OHSD [1]. We have recently demonstrated high 11β-OHSD mRNA expression and $NADP^+$-dependent bioactivity in several brain regions, including hippocampus, cortex and hypothalamus [11], in agreement with earlier observations [12, 13] and recent immunohistochemical findings [14]. Thus, the distribution of 11β-OHSD in brain may be appropriate to explain aspects of *in vivo* corticosteroid-receptor specificity. We have now used the fully-quantitative autoradiographic [^{14}C]2-deoxyglucose technique in conscious rats to investigate whether brain 11β-OHSD might be relevant *in vivo*.

EXPERIMENTAL

Studies were performed in intact male Sprague–Dawley rats (300 g); adrenalectomy was not performed as this leads to very widespread changes in local [^{14}C]2-deoxyglucose uptake that are only partly reversed by dexamethasone [15] (implying that non-corticosteroid effects of short-term adrenalectomy alter cerebral glucose utilization). Bilateral femoral arterial and venous cannulae were implanted under light halothane anaesthesia and the rats were allowed to recover, loosely restrained, for 3 h [16]. Rats were then injected with GE (Aldrich Chemicals) 5 mg s.c. in ethanol ($n = 5$) or ethanol vehicle ($n = 4$) at 0 min and again at 45 min. To examine the effects of 11β-OHSD inhibition in the presence of higher (stress-related) levels of B, further rats were injected with B (500 μg s.c. in ethanol) immediately after the second dose of GE ($n = 5$) or vehicle ($n = 4$). Local cerebral glucose utilization was measured using the fully-quantitative [^{14}C]2-deoxyglucose autoradiographic technique. Tracer was injected at 75 min and the animals killed at 120 min [17]. Autoradiograms were prepared from coronal brain sections (300 × 20 μm) and analysed densitometrically (Quantimet-970, Cambridge Instruments), as previously reported [16]. Local rates of glucose use were calculated using the operational equation for the method [17]. Sixty forebrain regions were examined; all areas showing significant changes and appropriate control regions are shown (Table 1). Statistical comparisons were performed by modified ANOVA [16]. Significance was set at $P < 0.05$. Values are mean ± SEM.

RESULTS

By the end of the 3 h recovery period following surgery the animals were not apparently distressed and plasma B was low (70 ± 7 nmol/l). Administration of GE (to inhibit 11β-OHSD) led to significant increases in metabolic activity

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Table 1. Effects of GE and/or B on [^{14}C]2-deoxyglucose utilization (μg/100 g·min) in conscious rats

Structure	Vehicle	GE	B	GE + B	F
Hypothalamus					
Preoptic area	51 ± 3	62 ± 5*	51 ± 1	61 ± 1*	5.79
Paraventricular nucleus	49 ± 4	52 ± 1	46 ± 1	55 ± 1*	5.75
Arcuate nucleus	47 ± 3	60 ± 1*	54 ± 4	67 ± 3**	7.20
Postlateral hypothalamus	48 ± 1	49 ± 3	51 ± 2	57 ± 2*	3.85
Posterior hypothalamus	49 ± 2	48 ± 1	44 ± 1	51 ± 2	2.35
Subfornical organ	79 ± 2	78 ± 2	77 ± 1	72 ± 2*	2.94
Parietal cortex					
Layer IV	92 ± 3	98 ± 5	98 ± 4	113 ± 2**	6.13
Layer VI	72 ± 5	82 ± 5	80 ± 5	75 ± 2	0.98
Frontal cortex	103 ± 4	103 ± 4	97 ± 5	100 ± 4	0.41
Hippocampus					
Dentate gyrus	42 ± 1	45 ± 3	42 ± 1	47 ± 3	1.04
CA1	45 ± 3	43 ± 2	43 ± 2	47 ± 2	1.17
CA3	56 ± 1	60 ± 2	57 ± 2	69 ± 1**	16.35
Molecular layer	79 ± 4	70 ± 4	69 ± 3	73 ± 4	1.21
Zona incerta	47 ± 2	53 ± 4	50 ± 1	66 ± 5**	5.24
Nucleus gelatinosus	97 ± 3	107 ± 8	91 ± 3	108 ± 4	3.51
Lateral habenula	92 ± 3	106 ± 9	93 ± 6	98 ± 7	0.92
White matter	20 ± 2	19 ± 2	24 ± 2	21 ± 1	2.62

*$P < 0.05$ and **$P < 0.01$ compared with control (ethanol vehicle alone) by ANOVA (F).

in the hypothalamic preoptic area (20%) and arcuate nuclei (26%), compared to vehicle injected controls (Table 1). Administration of exogenous B elevated plasma B levels to 603 ± 35 nmol/l at the time of [^{14}C]2-deoxyglucose injection (compared with 51 ± 6 nmol/l in controls at this time) but B injection alone (without GE) had no significant effect on local cerebral glucose use in any forebrain region (Table 1). Injection of GE in the presence of elevated B again led to significant increases in glucose utilization in the arcuate nucleus (43%) and hypothalamic preoptic area (20%), but in addition there was increased glucose use in the parietal cortex, layer IV (23%), CA3 hippocampus (23%), lateral hypothalamus (19%), paraventricular nucleus (12%) and zona incerta (40%). There was a small (9%) but significant fall in glucose use in the subfornical organ. The treatments had no effect on the overt behaviour of the rats and there were no significant changes or differences in blood pressure (monitored continuously), plasma glucose, pCO_2, pO_2 and pH during the study in any group.

DISCUSSION

Neither plasma B nor catecholamines [18] are elevated above non-stressed basal values by the [^{14}C]2-deoxyglucose method; at these concentrations B will largely occupy high-affinity MR rather than low-affinity GR. GE administration alone led to changes in glucose use only in the preoptic area and arcuate nuclei, presumably by exposing MR protected by 11β-OHSD to basal levels of B. The anterior hypothalamus/preoptic area is the site of action for aldosterone-mediated effects on central blood pressure regulation and saltwater homeostasis [2, 19]. In this region MR are aldosterone specific *in vivo* [3] and neurons show high 11β-OHSD mRNA expression [11]. Thus 11β-OHSD may modulate central corticosteroid effects on blood pressure and salt appetite. Certainly, lesions of the anteroventral third ventricle (including the preoptic area) prevent the development of mineralocorticoid or stress-induced hypertension [20, 21].

Administration of exogenous B elevated plasma B approximately 10-fold (to stress-related levels), which would be expected to occupy further MR and also GR. Despite this, B alone had no significant effect on glucose utilization in any brain region. This is surprising in view of the varied effects of B on mood, behaviour, neuroendocrine responses and neurochemical processes [1]. It is possible that the time course of these experiments was too short for glucocorticoid effects to be manifest, although positive effects on glucose use were found after GE pre-treatment.

In the presence of high levels of B, administration of GE led to widespread changes in glucose metabolism/function in the hypothalamus. The arcuate and paraventricular nuclei show high GR expression and 11β-OHSD may modulate hypothalamic B–GR interactions, analogous to that demonstrated in skin [22]. However, previous work has shown little change in cerebral glucose use after administration of dexamethasone [15]. Since this synthetic corticosteroid (which is not a substrate for 11β-OHSD) preferentially binds to GR, it seems unlikely that acute activation of GR is reflected in widespread changes in local cerebral glucose use. Furthermore, both MR and 11β-OHSD-like immunoreactivities have been described in these hypothalamic subregions [23] and the enzyme may thus be protecting MR from higher levels of B. Alternative interpretations of the effects of GE should also be considered. GE, though a potent inhibitor of brain 11β-OHSD [24], may have other central actions; it inhibits hepatic 5β-reductase and 3β-hydroxysteroid dehydrogenase [25] although this leads to accumulation of active aldosterone metabolites that would add to mineralocorticoid effects of 11β-OHSD inhibition. Alternatively, functional changes (in glucose use) may have occurred in regions only neuroanatomically connected to the primary site of action.

Hippocampus and cortex have high 11β-OHSD mRNA expression [11]. We found increased metabolic activity in hippocampus and parietal cortex when 11β-OHSD was inhibited in the presence of stress-mimicking levels of B. Since hippocampal 11β-OHSD bioactivity is attenuated by adrenalectomy and increased by high dose glucocorticoids [26], the absence of aldosterone selectivity of hippocampal MR in ligand binding studies may have resulted from their requirement for prior adrenalectomy. Furthermore, a proportion of hippocampal aldosterone binding cannot be displaced by excess B [2], and aldosterone and B have discrete actions at hippocampal MR [27], suggesting that a population of hippocampal MR may be protected by 11β-OHSD *in vivo*.

In summary, although the biological importance of 11β-OHSD activity remains to be determined, the current studies provide evidence that specific brain regions including the hippocampus and hypothalamus have functional enzyme *in vivo*.

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