Tissue-specific regulation of sialyltransferase activities in the rat by corticosteroids in vivo

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In this study we have examined the effects of corticosteroids on both the total sialyltransferase (sialyl-T) activity and on two individual isozymes in neural, hepatic, and renal tissues using an in vivo model system. Rats were adrenalectomised to deplete their endogenous stores of steroid hormones, and some subsequently received steroid replacement with dexamethasone or aldosterone. Adrenalectomy resulted in a significant decrease in total neural sialyl-T activity when compared with sham-operated animals in the four brain regions examined, indicating that total sialyl-T activity is normally under positive corticosterone control. The subsequent effects of exogenous corticosteroids exhibited regional specificity with the enzyme activities in the cortex, cerebellum, and brainstem being stimulated by both dexamethasone and aldosterone and enzyme activity in the hippocampus being stimulated by aldosterone alone. In general, the changes in total enzyme activity could be attributed to the α2,6 sialyl-T isozyme, although the changes in the cerebellum appeared to coincide with α2,3 sialyl-T activity. In the liver, adrenalectomy resulted in an increase in enzyme activity which was not altered by administration of corticosteroids. There were no changes in total renal sialyl-T activity in any of the four experimental groups although certain changes were observed at the level of individual sialyl-T isozymes. These results demonstrate that sialyl-T activity in certain tissues is under the control of corticosteroids and that this is both a tissue-specific and region-specific effect.

Key words: sialyltransferase/corticosteroid/glycoprotein/brain

Introduction

Sialic acid is a negatively charged sugar which is present primarily as a terminal group on the oligosaccharide groups of both glycolipids and glycoproteins. Because of its negative charge, the sugar is considered to have a particular influence on the structure of the backbone moiety. Sialic acid is transferred onto the newly synthesised protein by the sialyltransferase (sialyl-T) enzyme which is present in the trans-Golgi and the trans Golgi network (TGN). The sugar can be joined to other sugar residues in three major linkages, α2,3, α2,6, and α2,8, each being catalysed by an individual sialyl-T isozyme. The complexity of the sialyl-T family is increased by the fact that each isozyme catalyses the transfer of the sialic acid moiety to a specific acceptor sugar group (Lis and Sharon, 1993) and complex interactions between the individual isozymes have been proposed (Lee et al., 1989, 1990; Livingston et al., 1990). In addition, sialyl-T activity may also be autoregulated by the glycosylation state of the enzyme itself (Fast et al., 1993). To date, at least eight different sialyl-T isozymes have been isolated and cloned. Although the sialylation process is ubiquitous within the body, the expression of particular isozymes demonstrates a tissue specificity (Wen et al., 1992, Kitagawa and Paulson, 1993, 1994, Livingston and Paulson, 1993).

The control of enzyme expression and activity is multifactorial. The α2,6-sialyl-T isozyme is controlled in vitro in rat liver cells by the synthetic glucocorticoid dexamethasone (Wang et al., 1989, 1990; Bosshart and Berger, 1992). In addition, studies have demonstrated sialyl-T activity to be altered by cytokines in endothelial cells (Hanasaki et al., 1994) and the release of a soluble form of the enzyme from the liver may be induced as part of the acute phase response (Jamieson et al., 1993). The enzyme is also under oncogenic control in fibroblast cell lines (Easton et al., 1991; Le Marer et al., 1992; Delannoy et al., 1993). Furthermore, sialyl-T expression is developmentally regulated in neural tissue (Breen et al., 1987), in the intestine (Broquet et al., 1991), and in retinal tissue (Uehara et al., 1993). This developmental effect can be mirrored in vitro where certain cell lines exhibit a cell contact-dependent control of enzyme expression (Coughlan and Breen, 1995).

Corticosteroids are produced by the adrenal cortex and are a key component of the stress response (Sapolsky, 1992). They are not stored preformed, but rather they are synthesised and released as required, under the hierarchical control of the hypothalamic-pituitary-adrenal (HPA) axis (Seckl, 1995). The two types of corticosteroid receptors, mineralocorticoid (MR) and glucocorticoid (GR), which are members of the steroid hormone receptor gene superfamily, can be distinguished by their ligand affinities: glucocorticoid having the highest affinity for synthetic glucocorticoids such as dexamethasone, mineralocorticoid receptors having a much higher affinity for corticosterone and the physiological mineralocorticoid aldosterone (Miller et al., 1993). Under normal conditions, MR in the CNS are largely occupied while GR are unoccupied. The latter, however, become progressively occupied under stressful conditions (Reul et al., 1987). GR are widely expressed throughout the brain, while MR are found at low densities in most brain regions except the hippocampus and the septum (Reul and de Kloet, 1985).

Because of the pivotal role of sialoglycoproteins in cell and tissue function, agents which alter sialyl-T activity would be expected to have a major effect on cell functioning. While in vitro studies have demonstrated that dexa-
methasone alters total cellular sialyl-T activity in both neural (Coughlan and Breen, 1993) and hepatoma cell lines (Wang et al., 1989; Bosshart and Berger, 1992), the aim of this study was to examine the effects of corticosteroids on enzyme activity using an in vivo model system. Total sialyl-T activity was determined using asialofetuin as the predominant protein acceptor and included sialic acid in both α2,3 and α2,6 linkages to O- and N-linked oligosaccharide chains (Spro, 1960). The activities of individual isozymes were determined by a lectin-based assay system using the Maackia amurensis and Sambucus nigra lectins which recognise α2,3 and α2,6-linked sialic acid, respectively (Shibuya et al., 1987; Wang and Cummings, 1988).

**Results**

Significant total sialyl-T activity was detected in all of the tissues examined. The activity in the control (sham) animals was greatest in the liver with significantly lower levels in the kidney and the brain (Table 1). This could be attributed primarily to its high level of α2,6 sialyl-T activity. In the brain, regional specificity was observed with the highest total enzyme activity being observed in the cerebellum and the lowest in the cortex. However, the regional activities of the two individual isozymes showed a considerable variation. The α2,3 sialyl-T activity was highest in the hippocampus and lowest in the cortex, while the α2,6 sialyl-T activity was highest in the brainstem and lowest in the cerebellum.

Corticosteroids were shown to significantly affect sialyl-T enzyme activity in the brain. In three of the four regions examined (brainstem, cerebellum, and hippocampus), there was a significant decrease in total enzyme activity following adrenalectomy (Figure 1). Dexamethasone restored enzyme levels to control levels in all regions except the hippocampus. Aldosterone also restored enzyme levels to pre-adrenalectomised levels, and this effect was observed in all of the brain regions examined. These changes could be attributed to the α2,6 sialyl-T isozyme in the brainstem, cortex, and hippocampus (Figure 2b), but a similar trend was observed for the α2,3 sialyl-T isozyme in the cerebellum (Figure 2a). It could be noted that there was a general trend towards an antagonism between the two isozymes in that a decrease in the activity of one isozyme was accompanied by an increase in the activity of the other.

Adrenalectomy caused a twofold increase in total liver sialyl-T activity, with this level of enzyme activity being maintained following treatment with either dexamethasone or aldosterone (Figure 3a). There was no significant difference between the enzyme activity in the sham-operated animals, and data available for unoperated control animals (data not shown) indicated that the operational procedure is unlikely to have a significant effect on the enzyme activity. When individual isozyme activity was determined, there was a slight but significant decrease in sialyl-T activities following adrenalectomy with a significant increase following dexamethasone or aldosterone treatment (Figure 3b,c). These changes were particularly visible for the α2,6 sialyl-T isozyme where there was an 8-fold increase in activity following dexamethasone treatment.

While adrenalectomy or subsequent drug treatment had no effect on total kidney enzyme activity, there was a slight decrease in both α2,3 and α2,6 sialyl-T activities following adrenalectomy which was reversed by treatment with dexamethasone (Figure 4). Aldosterone did not appear to have any effect on the activity of either isozyme.

**Discussion**

Previous studies have examined the induction of sialyl-T activity by corticosteroids in both hepatoma and neural cell lines in vitro (Wang et al., 1989; Coughlan and Breen, 1993; Kleene and Berger, 1993). The aim of this study was to examine the physiological relevance of this induction by investigating the effect of corticosteroids in vivo on total tissue sialyl-T activity and the activities of two constituent isozymes, α2,3 and α2,6 sialyl-T. For this purpose, we used an animal model where serum levels of corticosteroids were carefully controlled by adrenalectomy (Farman et al., 1994), and then the individual effects of the GR agonist dexamethasone and the mineralocorticoid receptor agonist aldosterone were examined.

The basal (sham) enzyme activities varied significantly between brain regions with the highest total sialyl-T activity in the cerebellum and the lowest in the cortex. This situation was further compounded by a regional variation in the activities of two constituent isozymes. This may reflect the differential expression of sialoglycoprotein species that have been observed in the brain (Coughlan et al., 1996). In all four brain regions examined, adrenalectomy resulted in a decrease in total sialyl-T activity, suggesting that neural sialyl-T levels are maintained by the levels of circulating corticosteroids under normal conditions. This change could be generally attributed to the α2,6 isozyme in the brainstem, cortex, and hippocampus but appeared to be due to a slight decrease in α2,3 activity in the cerebellum. These corticosteroid-dependent changes in α2,6 sialyl-T activity are in good agreement with those observed in

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### Table I. Sialyl-T specific activities in homogenates from liver, kidney, and brain regions from control (sham) animals (n = 5)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total sialyltransferase pmol NeuNAc/mg/h</th>
<th>α2,3 sialyltransferase μg fetuin/mg/h</th>
<th>α2,6 sialyltransferase μg fetuin/mg/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>78.83 ± 8.01</td>
<td>1.53 ± 0.16</td>
<td>8.24 ± 1.24</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.68 ± 0.76</td>
<td>0.80 ± 0.07</td>
<td>0.68 ± 0.06</td>
</tr>
<tr>
<td>Brain–Parietal cortex</td>
<td>2.26 ± 0.42</td>
<td>0.35 ± 0.01</td>
<td>1.10 ± 0.47</td>
</tr>
<tr>
<td>Brain–Hippocampus</td>
<td>3.46 ± 0.54</td>
<td>1.44 ± 1.05</td>
<td>1.15 ± 0.41</td>
</tr>
<tr>
<td>Brain–Brainstem</td>
<td>3.51 ± 0.33</td>
<td>1.06 ± 0.09</td>
<td>2.41 ± 0.36</td>
</tr>
<tr>
<td>Brain–Cerebellum</td>
<td>5.56 ± 0.93</td>
<td>1.20 ± 0.49</td>
<td>0.32 ± 0.05</td>
</tr>
</tbody>
</table>
other tissues (Wang et al., 1989, 1990). While there was only a slight decrease in the total enzyme activity in the cortex, which did not reach levels of significance, the adrenalectomy-induced decrease was particularly pronounced in both the cerebellum and hippocampus (Figure 1). These differences in sialyl-T activity and their susceptibilities to corticosteroids may be accounted for by variable basal levels of total enzyme activity in the different brain regions. The fact that total enzyme activity values are very similar in all four brain regions following adrenalectomy suggests that the regional differences in sialyl-T activity observed in the sham-operated animals may be due, at least in part, to a differential susceptibility of the enzyme activity to the stimulatory activity of circulating corticosteroids.

Of particular interest were the contradictory effects of the corticosteroids on the activities of the α2,3 and α2,6 sialyl-T isozymes. In general, a decrease in the activity of one isozyme was accompanied by an increase in the other. This suggests that the two isozymes may compete for the one single site (e.g., a terminal galactose residue in N-
Fig. 2. a. α2,3 sialyl-T; and b. α2,6 sialyl-T activities in brainstem, cerebellar, cortical, and hippocampal brain homogenates from the four animal groups. * p < 0.05 as calculated using an unpaired Student’s t test (n = 5).
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Fig. 3. (a) Total sialyl-T, (b) α2,3 sialyl-T, and (c) α2,6 sialyl-T activities in liver homogenates from the four animal groups. *, p < 0.05, and **, p < 0.005 as calculated using an unpaired Student's t test (n = 5).

Fig. 4. (a) Total sialyl-T; (b) α2,3 sialyl-T; and (c) α2,6 sialyl-T activities in kidney homogenates from the four animal groups. *, p < 0.05, and **, p < 0.005 as calculated using an unpaired Student's t test (n = 5).
linked oligosaccharides) with the enzyme which has the highest activity transferring a greater amount of sialic acid. Such a complex interaction between sialyl-T isozymes has, in fact, been previously proposed (Lee et al., 1989). Furthermore, we have also demonstrated parallel changes in the expression pattern of sialoglycoprotein by lectin blot analysis following the steroid-induced alterations in enzyme activity (Coughlan et al., 1996). The expression pattern of the individual glycoprotein species varied considerably between the different brain regions with a complex interrelationship also existing between the ratios of α2,3 and α2,6 sialoglycoprotein expression.

In three of the brain regions: the cortex, cerebellum, and brainstem, administration of dexamethasone following adrenalectomy served to restore total enzyme levels to those of the sham-operated group and this trend was also observed for the activity of the individual isozyme species that had been affected by adrenalectomy (Figures 1, 2). This was not observed, however, in the hippocampus where enzyme levels remained at the level of the adrenalectomised group. However, the administration of aldosterone significantly increased total enzyme activity in all four brain regions. Such a trend was also observed for the activity of the hippocampal α2,6 sialyl-T which was also stimulated by aldosterone (Figure 2b). In the cerebellum, cortex, and brainstem, where sialyl-T activity was stimulated by dexamethasone, aldosterone appeared to be more potent in inducing enzyme activity as levels were generally raised to slightly above sham levels, with these reaching significance in the brainstem. The fact that probably supraphysiological doses of either steroid in general did not raise enzyme levels significantly above sham values suggests that under normal circumstances, the total sialyl-T activity in the brain is possibly close to maximum levels and cannot be generally induced further, at least by corticosteroids. Furthermore, the susceptibility of enzyme activity to induction by individual corticosteroids is region-specific with the hippocampal form of the enzyme resistant to the actions of glucocorticoids. The fact that dexamethasone does not induce sialyl-T in the hippocampus cannot be explained by a lack of glucocorticoid receptors in the region as the hippocampus is rich in this receptor class (Seckl, 1995).

While corticosteroids appear to have a similar effect on sialyl-T activity in neural cells in vitro as has previously been reported in vivo using neural cell model systems (Coughlan and Breen, 1993), the effects of corticosteroids on total hepatic sialyl-T did not mirror those observed in hepatoma cell lines (Wang et al., 1989, 1990; Bosshart and Berger, 1992). Adrenalectomy resulted in a twofold increase in total enzyme activity, an increase which was maintained following treatment with either dexamethasone or aldosterone (Figure 3). While, initially, this could suggest a possible negative regulation of basal hepatic sialyl-T activity by corticosteroids with depletion of endogenous steroids resulting in an increase in enzyme activity, subsequent treatment with either dexamethasone or aldosterone had no effect on enzyme induction. It is likely, therefore, that other factors may be involved in the enzyme induction. Although previous studies have demonstrated an induction of hepatic sialyltransferase activity as part of the acute phase response, (Jamieson et al., 1993; Hanasaki et al., 1994), it is unlikely that this a similar mechanism is involved in this situation as any inflammatory reaction associated with the adrenalectomy procedure would be controlled by the sham group of animals which underwent identical surgery (the opening of the peritoneal cavity) without the removal of the adrenal glands. Furthermore, the enzyme activities in the sham-operated animals were similar to data available for unoperated animals indicating that the operational procedure is unlikely to have a significant effect on enzyme activity. It is possible that hepatic sialyl-T activity is under the negative control of other factors released by the adrenal medulla such as catecholamines or adrenal androgens. There is no evidence available to date, however, suggesting a role for these agents in the control of sialyl-T expression. However, the pattern of activities of the α2,3 and α2,6 sialyl-T isozymes varied from total sialyl-T activity. For both isozymes, there was a significant decrease in enzyme activity following adrenalectomy followed by a stimulation to levels significantly above normal following treatment with dexamethasone or aldosterone. While corticosteroid stimulation of α2,6 sialyl-T has previously been reported, we show here that the α2,3 sialyl-T isozyme is also under corticosteroid control, albeit to a much lesser extent.

The increase in the total sialyl-T activity following adrenalectomy cannot be attributed to either the α2,3 or α2,6 sialyl-T isozymes hence the increase may be due to the induction of other sialyl-T isozyme species which are not detected by the lectin-based assay system. In addition, however, while the total sialyl-T assay primarily measures sialic acid transferred to the asialofetuin acceptor, endogenous transfer to membrane glycoproteins will also be detected as all of the assay protein content is precipitated by trichloroacetic acid. In the lectin based assays, the asialofetuin acceptor is immobilised to the solid phase and only the sialic acid bound to this acceptor is quantified as the endogenous components are removed after the assay by washing. The endogenous glycoproteins may thus provide additional acceptor sites which may be altered following changes in the activities of other glycosyltransferase enzymes. Thus, while the α2,3 and α2,6 sialyl-T assays are a good measure of enzyme specific activity, total sialyl-T may be a more accurate measure of total tissue sialylation potential and is, indeed, in good agreement with total tissue sialic acid content (Coughlan and Breen, unpublished observations).

In contrast to both the liver and brain, corticosteroids appear to have no effect on total sialyl-T activity in the kidney (Figure 3). Indeed, the fact that the adrenalectomy procedure itself did not alter enzyme activity suggests that there are differential responses of sialyl-T in individual tissues to corticosteroids which may reflect the mechanisms controlling tissue-specific glycosylation. There was, however, a decrease in the activities of the individual α2,3 and α2,6 sialyl-T isozymes which was reversed upon dexamethasone treatment. The contradiction between total sialyl-T and individual sialyl-T isozyme activities is similar to that observed in the liver and may again reflect changes in the availability of endogenous acceptor sites.

This study has demonstrated that sialyl-T isozyme activity may be modulated by corticosteroid levels in vivo, and this may have a significant effect on cell function. Because of the number of CNS conditions (depression and age-related neurodegeneration) in which variations in serum glucocorticoid levels have been implicated, it will be of great interest to examine the functional changes asso-
associated with steroid-induced modulation of protein glycosylation.

Materials and methods

Animals

Four groups of rats were used with five animals in each group. The control group was sham operated with the other three being adrenalectomised, all under halothane anaesthesia (Farman et al. 1994). The adrenalectomised animals were then treated as follows: group A received vehicle (20% ethanol:saline) subcutaneously, group B were treated with dexamethasone (0.2 mg/kg), and group C were treated with aldosterone (0.05 mg/kg). The animals were then sacrificed 24 h posttreatment.

Tissue preparation

Following sacrifice, the brain, liver, and kidneys were removed from the animals. The brains were then further dissected into four regions: the hippocampus, cortex (parietal), cerebellum, and brainstem. These were snap-frozen and maintained at ~70°C until use. The tissues were homogenised in 10 volumes of ice-cold homogenisation buffer (50 mM tris buffer, pH 6.5, containing 150 mM NaCl, 5 mM EDTA, 1 μg/ml aprotonin, and 1 mM phenylmethylsulfonylfluoride) using a Teflon homogeniser, and the homogenate samples were stored in aliquots at ~20°C prior to analysis. Upon defrosting, the samples were briefly sonicated before use to prevent tissue clumping. The protein content of the samples was determined by the method of Lowry et al. (1951) using the Folin phenol reagent.

Total sialyltransferase assay

Total tissue sialyltransferase was assayed according to the method of Breen and Regan (Breen and Regan, 1986) using cytidine 5-monophosphate-4,5,6,7,8,9-14C-acetyleneuraminic acid (CMP-14C-NeuNAc, Radiochemical Centre, Armersham; specific activity 293 mCi/mmol) as the sugar donor and asialofetuin (Sigma) as the exogenous acceptor. The transferred sialic acid was determined by liquid scintillation counting and the results expressed as picomoles of sialic acid transferred per milligram of protein per hour.

Sialyltransferase isozyme assays

The activities of the α2,3 and α2,6 sialyl-T isozymes were determined using a lectin-based microtiter plate assay system as previously described (Maguire et al. 1994). The transfer of sialic acid from the activated CMP-NeuNAc donor to the asialofetuin acceptor in α2,6 and α2,3 linkages was detected using the Sambucus nigra (SNA) and Maackia amurensis (MAA) lectins, respectively, with boiled blanks serving as controls. The assays were standardised using the fetuin sialoglycoprotein (SGP).

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