Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone

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Gomez-Sanchez, Elise P., and Celso E. Gomez-Sanchez. Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone. Am. J. Physiol. 263 (Endocrinol. Metab. 26): E1125–E1130, 1992.—The apparent mineralocorticoid excess syndrome of patients ingesting large amounts of licorice or its derivatives is thought to be caused by the antagonism by these compounds of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD inactivates cortisol and corticosterone, allowing the more abundantly produced glucocorticoids access to the mineralocorticoid receptor (MR) in the kidney, where they act as mineralocorticoids. We have found that the inhibition of both glycyrrhizic acid, an active principle of licorice, and carbenoxolone, a synthetic analogue, into a lateral ventricle of the brain [intracerebroventricular (icv)] of a rat, at a dose less than that which has an effect when infused subcutaneously, produces hypertension. Furthermore, the hypertension produced by the oral administration of carbenoxolone or glycyrrhizic acid is blocked by the icv administration of RU 28318, an MR antagonist, at a dose below that which has an effect on blood pressure when infused subcutaneously. While the oral administration caused saline polydipsia and polyuria typical of chronic systemic mineralocorticoid excess, the icv licorice derivatives produced hypertension without affecting saline appetite. Sensitizing the rats to mineralocorticoid hypertension by renal mass reduction and increasing salt consumption was not necessary for the production of hypertension. These findings provide additional evidence for a central role in blood pressure control by mineralocorticoids that is distinct from their renal effects. They also suggest that more is involved in licorice-induced hypertension than only inhibition of 11β-HSD.

hypertension; licorice; mineralcorticoids; RU 28318; steroid 11β-hydroxysteroid dehydrogenase

ALDOSTERONE acts through type I receptors, or mineralocorticoid receptors (MR), in the kidney to produce sodium retention and potassium and hydrogen ion excretion. The MR is widely distributed and is present in the colon, parotid, vasculature, and, in particular, specific areas of the brain (5, 13). The affinity of isolated MR from various sources, including expressed MR cDNA in COS cells, is similar for aldosterone, cortisol, and cortisol (3, 4, 16). MR, regardless of the source, are physicochemically identical (16, 32), and appear to be a product of the same cDNA (3). Corticosterone and cortisol normally do not act as mineralocorticoids in the kidney in vivo. Specificity, originally thought to be intrinsic to the receptor, has been shown to be conferred extrinsically by corticosterone/cortisol-binding globulin (CBG), which reduces free circulating glucocorticoid available to the receptor, and by 11β-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD reversibly converts corticosterone and cortisol to the inactive 11-dehydrocorticosterone and cortisone (7, 9, 12). The location of the 11β-HSD enzyme has been controversial. It appears that 11β-HSD is expressed in some mineralocorticoid target cells along with the MR, thus serving as an autocrine control, as well as in cells proximate to MR-containing cells, serving a paracrine function (6, 9, 21, 24).

Under normal conditions, most MR in the rat brain are almost fully occupied by corticosterone, while occupation of the type II receptor, or glucocorticoid receptor (GR), for which corticosterone has less affinity, is less complete and follows the circadian rhythm of glucocorticoid levels (7). It has been suggested that the occupation of the MR in the brain, particularly in the hippocampus, by corticosterone at low, physiological serum levels is possible because CBG does not penetrate the blood-brain barrier (7, 9) and because the activity of 11β-HSD in this organ is negligible (9, 12). However, in situ hybridization techniques have demonstrated the presence of 11β-HSD in the brain (19), as well as the kidney. Whether 11β-HSD is bioactive in any, all, or only specific parts of the brain is controversial (9, 19, 21). There are different tissue-specific forms and regional activity of the 11β HSD enzyme (20) that may account for the apparent “glucocorticoid-selective” MR in some parts, particularly the hippocampus, of the brain, in contrast to the “aldosterone-prefering” MR in the anterior hypothalamus (7, 18). Seckl et al. (27) have reported that 11β-HSD inhibition by glycyrrhetinic acid in vivo in rats increased 2-deoxy-[14C]glucose use in those areas of the brain where 11β-HSD mRNA expression has been documented. Corticosterone and aldosterone have different actions in some areas of the brain, even though both are thought to be acting with the same affinity and through the same receptor. Aldosterone antagonizes important central nervous system (CNS) effects of corticosterone (7, 26); corticosterone blocks the hypertension induced by the intracerebroventricular (icv) infusion of aldosterone (13, 15).

Apparent mineralocorticoid excess is a rare hypertensive syndrome in which patients have all of the manifestations of excessive production of mineralocorticoids, including hypokalemia, but steroid measurements are normal or low. The defect has been identified as a deficiency in 11β-HSD (11, 28, 30, 31). The pseudohyperaldosteronism, including hypokalemia and low-renin hypertension, produced by excessive licorice consumption and the treatment of peptic ulcers with licorice derivatives or their synthetic analogues has been attributed to the inhibition of this enzyme, allowing the more abundant circulating cortisol/corticosterone access to the MR in the kidney (9). Licorice derivatives and the synthetic analogue carbenoxolone have been used to study the mechanisms responsible for the syndrome of apparent mineralocorticoid excess, as well as the extrinsic factors conferring apparent ligand specificity to the MR (8, 10, 22). We herein describe studies of the central and
systemic effects of the icv, subcutaneous (sc), and oral administration of glycyrrhizic acid, a derivative of licorice, and carbenoxolone, a synthetic analogue, on the blood pressure using the specific MR antagonist RU 28318 (14) to inhibit the MR.

METHODS

Cannulas were placed into the right lateral cerebral ventricles of male outbred Sprague-Dawley rats weighing 180-200 g, using aseptic surgical technique under a combination of fentanyl and droperidol (Innovar-Vet, Pitman-Moore). 0.01 ml/100 g body wt sc, as preanesthetic and isoflurane anesthesia, and pumps of the same lot were used throughout the experiment to ensure consistency. Carbenoxolone, RU 28318, and corticosterone were dissolved in cerebrospinal fluid (CSF) or 0.86% NaCl with 10% propyleneglycol for icv and sc infusion. A potassium gluconate solution that delivered the same amount of K+ as the RU 28318 solution was used as control for the mineralocorticoid antagonist experiments (14). Reagents were purchased from Sigma, except for the RU 28318, which was a gift from Roussel (Romaineville, France). All solutions were made and sterilized by filtration through 0.2-μm filters (Acrodisc 13, Gelman Scientific) immediately before filling and implanting the pumps. Oral carbenoxolone or glycyrrhizic acid was administered individually twice a day as 0.1 or 0.2 ml of a slurry mixed in corn syrup that the rats accepted readily. Indirect systolic blood pressures (ITTC, Woodhills, CA) and weights were measured twice a week before starting treatment as described previously (13). Twenty-four- or forty-eight-hour urine volumes were measured once a week in a stainless steel rat metabolism cage.

Effect of icv administration of carbenoxolone: dose response. Carbenoxolone was infused icv at a rate of 0.3, 1.0, and 3.0 μg/h and sc at a rate of 3.0 μg/h into intact rats provided with 0.9% saline to drink ad libitum.

Effect of icv administration of carbenoxolone and corticosterone. Carbenoxolone was infused icv at a rate of 5.0 μg/h and corticosterone at a rate of 20 ng/h, alone and together. Two types of experiments were done. For one, the rats were intact and drank tap water ad libitum. For the other, the right kidneys were removed and the rats drank 0.9% saline ad libitum to be comparable to the classical maneuvers used to amplify mineralocorticoid hypertension.

Effect of oral administration of carbenoxolone with and without icv RU 28318. Carbenoxolone was administered orally in corn syrup 45 mg/kg twice daily for 10 days and increased to 90 mg/kg twice daily for the next 4 days to ascertain that the hypertensive effect was maximal; the control rats received corn syrup orally. RU 28318 was infused icv at 1.1 μg/h in one-half of the animals receiving carbenoxolone; the other animals received a potassium gluconate solution to supply the equivalent amount of K+ icv. We have previously shown that 1.1 μg/h RU 28318 icv has no intrinsic effect on the blood pressure but protects the rat from the hypertension of systemic mineralocorticoid excess, while being well below the dose required to affect on the blood pressure when infused sc (13, 14). The rats were intact and drank tap water ad libitum.

Effect of oral administration of glycyrrhizic acid with and without RU 28318. The effects of both glycyrrhizic acid and carbenoxolone were studied because of evidence that carbenoxolone may have a larger range of effects, including the inhibition of 11-oxoreductase, than does glycyrrhizic acid (29).

Glycyrrhizic acid was administered orally in corn syrup 35 mg/kg twice daily for 14 days. RU 28318 was infused icv and sc at 1.1 μg/h in two of three glycyrrhizic acid groups; the other glycyrrhizic acid animals received a potassium gluconate solution icv to supply the equivalent amount of K+ icv. Another group received corn syrup orally and the potassium gluconate solution icv. The rats were intact and drank tap water ad libitum.

Animals were killed at the end of the studies by CO2 narcosis and asphyxiation. Autopsies, including dye infusions to check cannula placement, were done at the conclusion of the study, and data from any animal in which there was doubt about the delivery of the solutions or which had evidence of illness causing undue stress were eliminated from the experiment. At the time of the biweekly pump changes, if the catheter was found to be disconnected from the pump or cannula, the data from the preceding two weeks were discarded and the animal eliminated from the study. We started with 8-10 animals per group so that the groups were never reduced to fewer than 7 animals by the end of the experiment. Data were compared by analysis of variance and the Dunnett t and Fisher PLSD tests (StatView 512+, BrainPower, Calabazas, CA).

RESULTS

Carbenoxolone, 3 μg/h administered icv to intact rats drinking 0.9% saline ad libitum, increased the blood pressure of rats significantly (P < 0.01) within 3 days and was maximal by day 5 (Fig. 1). There was no significant change in the blood pressure of rats receiving 0.3 μg/h CSF, or 1 μg/h carbenoxolone icv or 3 μg/h carbenoxolone sc over 14 days. No significant difference was found in rate of weight gain or 24-h urine volume between any groups in the icv studies. In separate studies it was found that doses of carbenoxolone >5 μg/h resulted in precipitation of the drug in the pump and cannulas.

The icv infusion of corticosterone at 20 ng/h, a dose known to inhibit the hypertension produced by the icv infusion of aldosterone (15) while having no effect in and of itself, did not significantly blunt the increase in blood pressure produced by icv carbenoxolone, nor did it have any effect on the blood pressure by itself (Fig. 2). There was no difference in urine volume or weight gain between groups in the same experiments. Removing one kidney and giving saline to drink did not alter the hypertension.
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Fig. 2. Effect on indirect systolic blood pressure of icv infusion of carbenoxolone at 5.0 \( \mu \)g/h and corticosterone at 20 ng/h, alone and together, in nonsensitized rats (A; intact and drinking tap water ad libitum) compared with sensitized rats (B; one kidney removed and drinking 0.9% saline ad libitum). \( \beta \), 11\( \beta \)-hydroxysteroid dehydrogenase. * \( P < 0.05 \), ** \( P < 0.01 \).

Fig. 3. Effect on indirect systolic blood pressure of oral administration of carbenoxolone in corn syrup at 45 mg/kg twice daily for 10 days, then 90 mg/kg twice daily for the next 4 days, while receiving an icv infusion of either RU 28318 at 1.1 ng/h or vehicle, in intact rats drinking tap water ad libitum.

Fig. 4. Effect on 24-h urine volume of oral administration of carbenoxolone in corn syrup at 45 mg/kg twice daily for 10 days, then 90 mg/kg twice daily for the next 4 days, while receiving an icv infusion of either RU 28318 at 1.1 ng/h or vehicle, in intact rats drinking tap water ad libitum.

The blood pressure of intact rats drinking water and receiving oral carbenoxolone at 45 mg/kg twice daily increased significantly within 6 days from 105 mmHg to a plateau of 127 mmHg (Fig. 3). Doubling the dose to 90 mg/kg twice daily did not further increase the blood pressure. The icv infusion of 1.1 \( \mu \)g/h RU 28318 completely prevented the increase in blood pressure. We have shown in multiple studies, including those described below using glycyrrhizic acid instead of carbenoxolone, that the sc infusion of 1.1 \( \mu \)g/h RU 28318 is too low to affect the blood pressure. We have also reported that the icv infusion of the antagonist at three times this dose has no effect on the blood pressure of normal animals (14). The blood pressure in the animals receiving the icv control solution returned to normal within 3 days of discontinuing the oral administration of carbenoxolone. Orally administered carbenoxolone doubled the urine volume; this increase in urine volume was not prevented by the icv administration of the mineralocorticoid antagonist, which abolished the hypertension (Fig. 4). There was no difference in weight gain between groups.

The oral administration of glycyrrhizic acid at 35 mg/kg twice daily also significantly increased the blood pressure of intact rats drinking tap water. The icv infusion of 1.1 \( \mu \)g/h RU 28318 prevented the rise in blood pressure (Fig. 5). There was no difference in weight gain between groups.

DISCUSSION

The importance of the CNS in the development of mineralocorticoid hypertension has been well documented (5, 13). MR are found in the hippocampus, amygdala, lateral septum, and hypothalamus, particularly in the periventricular regions, areas known to be or suspected of being important in the regulation of adrenocorticotropic hormone (ACTH) release, arousal, fluid and fluid osmolality equilibrium, and the maintenance of normal blood pressure. The chronic icv infusion of aldosterone at a dose two orders of magnitude less than that necessary to produce hypertension when infused sc has been reported to produce hypertension in rats and dogs (21). The icv infusion of the mineralocorticoid antagonist RU 28318, at doses that have no effect on the blood pressure when given icv alone and that are ineffective as
an antagonist when administered sc, blocks the hypertension of both icv and systemic administration of aldosterone and the sc infusion of deoxycorticosterone acetate. The systemic, but not icv, aldosterone hypertension is associated with a chronically increased urine volume indicative of saline polydypsia/polyuria. The icv infusion of the antagonist prevents the rise in pressure produced by the systemic administration of aldosterone without preventing the associated polydypsia/polyuria (13, 14). These findings suggest distinct mineralocorticoid effects in the brain and kidney.

In the studies reported herein, the icv, but not sc, infusion of 3 µg/h carbenoxolone produced hypertension, implying that the site of action is in the brain. The hypertension produced by the oral and icv administration of carbenoxolone or glycyrrhizic acid resembles that of chronic systemic and icv aldosterone infusion in the amplitude of the increase in blood pressure and the effectiveness of mineralocorticoid receptor blockade by icv RU 28318 (13, 14). In addition, as with aldosterone, an increase in urine volume occurred only with the systemic, and not icv, administration of hypertensinogenic amounts of both licorice compounds. Blocking the hypertension of animals receiving oral carbenoxolone with the icv infusion of RU 28318 at doses too low to be effective when infused sc did not reduce their increase in urine volume. Classically mineralocorticoid-salt hypertension is associated with an initial retention of sodium and water followed by an "escape" from further retention and the establishment of a new equilibrium at a higher overall fluid volume. Polydypsia/polyuria may persist after the water retention phase, assuming it occurred, after the renal mass and increase sodium consumption and because in the model of central mineralocorticoid hypertension, equihypertensinogenic doses of icv aldosterone in non-sensitized rats were nine times that of sensitized rats (13).

However, if 11β-HSD were active in the brain, and it was inhibited by carbenoxolone, previous studies from our laboratory suggest that the resulting accumulation of corticosterone would not be expected to increase blood pressure. An additional difference between the icv aldosterone and icv carbenoxolone models is that the icv infusion of corticosterone, at a dose that would have been expected from our previous work to antagonize the icv aldosterone model, had no effect on the blood pressure of rats receiving icv carbenoxolone. It is assumed that the inhibitory action of icv corticosterone on icv aldosterone hypertension is mediated by the MR because RU 26988, a selective GR agonist, had no effect when infused alone or in combination with aldosterone (15). While most reported studies indicate that carbenoxolone does not affect the mineralocorticoid activity of aldosterone (25), others suggest that it enhances the sodium retention produced by aldosterone and 11-deoxycorticosterone (23). Glycyrrhetinic acid has been found to inhibit the hepatic 5α-reductase and 3β-HSD but not the 5α-reductase or 3α-HSD. Another proposed mechanism for the enhancement of mineralocorticoid activity by licorice derivatives is the accumulation of aldosterone, deoxycorticosterone, and 11-deoxycorticosterone and their biologically active 5α-dehydro derivatives due to the inhibition of the 5α-reductase and 3β-HSD enzymes, as well as of glucocorticoids due to 11β-HSD inhibition (17).

Patients with apparent mineralocorticoid excess appear to be deficient in 11β-dehydrogenase but not 11-oxoreductase enzyme activity (30). While it has been assumed that 11β-HSD is an enzyme complex consisting of an 11β-dehydrogenase and a distinct 11-oxoreductase (9, 22, 30), a rat cDNA has been cloned and expressed as a single enzyme that interconverts cortisol/corticosterone to cortisone/11-dehydrocorticosterone (1). It has been reported that glycyrrhizic acid and carbenoxolone are not identical in their clinical activities and that glycyrrhizic acid inhibits the conversion of cortisol/corticosterone from 7 to 11 days to become significant (13), while icv carbenoxolone hypertension was evident in 3–6 days. Considering the relatively long delay of onset, that of days rather than minutes or hours, it seems unlikely that this difference is due to a more rapid passage of the licorice compounds across the blood-brain barrier; it probably reflects a more basic difference in the mechanism of action. Removing one kidney and giving saline to drink did not exacerbate the hypertension produced by icv carbenoxolone. This was surprising because the classical way to amplify mineralocorticoid hypertension is to reduce renal mass and increase sodium consumption and because in the model of central mineralocorticoid hypertension, equihypertensinogenic doses of icv aldosterone in non-sensitized rats were nine times that of sensitized rats (13).

Glycyrrhizic acid and carbenoxolone are not thought to act as agonists at the receptor level because their affinity for the MR is negligible (2). They are presumed to work by inhibiting 11β-HSD, thereby removing the protection of the MR from corticosterone and allowing it to act as a mineralocorticoid (12). However, if 11β-HSD were active in the brain, and if it were inhibited by carbenoxolone, previous studies from our laboratory suggest that the resulting accumulation of corticosterone would not be expected to increase blood pressure. An additional difference between the icv aldosterone and icv carbenoxolone models is that the icv infusion of corticosterone, at a dose that would have been expected from our previous work to antagonize the icv aldosterone model, had no effect on the blood pressure of rats receiving icv carbenoxolone. It is assumed that the inhibitory action of icv corticosterone on icv aldosterone hypertension is mediated by the MR because RU 26988, a selective GR agonist, had no effect when infused alone or in combination with aldosterone (15).
to cortisone/11-dehydrocorticosterone unidirectionally, while carbenoxolone inhibits both the dehydrogenase and reductase directions (29). In our studies, the activity of glycyrrhizic acid and carbenoxolone were similar.

There is evidence for yet another mechanism of action of carbenoxolone. The MR is either missing or defective in patients with pseudohypoaldosteronism. Funder (10) has reported that the administration of carbenoxolone of carbenoxolone. The MR is either missing or defective function of the glucocorticoid, causing it to produce the tension by “recruiting” GR and/or corticosterone-prefering” MR. If carbenoxolone were producing hyperten-

The yin-yang relationship of the two classes of corticosteroids in their central effects on blood pressure, because the receptors were already sur-

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