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LIMITED BRAIN DIFFUSION OF THE GLUCOCORTICOID RECEPTOR AGONIST RU28362 FOLLOWING I.C.V. ADMINISTRATION: IMPLICATIONS FOR I.C.V. DRUG DELIVERY AND GLUCOCORTICOID NEGATIVE FEEDBACK IN THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS

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Abstract—The experiments described herein present a method for tracking diffusion of the glucocorticoid receptor agonist RU28362 in brain following i.c.v. drug administration. A useful property of glucocorticoid receptor is that it is primarily cytoplasmic when unbound and rapidly translocates to the nucleus when bound by ligand. Thus, removal of endogenous glucocorticoids by adrenalectomy allows us to identify brain regions with activated glucocorticoid receptor after i.c.v. glucocorticoid receptor agonist treatment by examining the presence or absence of nuclear glucocorticoid receptor immunostaining. We have previously demonstrated that an i.p. injection of 150 μg/kg RU28362 1 h prior to restraint stress is sufficient to suppress stress-induced hypothalamic–pituitary–adrenal axis hormone secretion [Ginsberg AB, Campeau S, Day HE, Spencer RL (2003) Acute glucocorticoid pretreatment suppresses stress-induced hypothalamic–pituitary–adrenal axis hormone secretion and expression of corticotropin-releasing hormone hnRNA but does not affect c-fos mRNA or fos protein expression in the paraventricular nucleus of the hypothalamus. J Neuroendocrinol 15:1075–1083]. We report here, however, that in rats i.c.v. treatment with a high-dose of RU28362 (1 μg) 1 h prior to stressor onset does not suppress stress-induced hypothalamic–pituitary–adrenal axis activity. We then performed a series of experiments to examine the possible differences in glucocorticoid receptor activation patterns in brain and pituitary after i.c.v. or i.p. treatment with RU28362. In a dose-response study we found that 1 h after i.c.v. injection of RU28362 (0.001, 0.1 and 1.0 μg) glucocorticoid receptor nuclear immunoreactivity was only evident in brain tissue immediately adjacent to the lateral or third ventricle, including the medial but not more lateral portion of the medial parvocellular paraventricular nucleus of the hypothalamus. In contrast, i.p. injection of RU28362 produced a uniform predominantly nuclear glucocorticoid receptor immunostaining pattern throughout all brain tissue. I.c.v. injection of the endogenous glucocorticoid receptor agonist, corticosterone (1 μg) also had limited diffusion into brain tissue. Time-course studies indicated that there was not a greater extent of nuclear glucocorticoid receptor immunostaining present in brain after shorter (10 or 30 min) or longer (2 or 3 h) intervals of time after i.c.v. RU28362 injection. Importantly, time-course studies found that i.c.v. RU28362 produced significant increases in nuclear glucocorticoid receptor immunostaining in the anterior pituitary that were evident within 10 min after injection and maximal after 1 h. These studies support an extensive literature indicating that drugs have very limited ability to diffuse out of the ventricles into brain tissue after i.c.v. injection, while at the same time reaching peripheral tissue sites. In addition, these studies indicate that significant occupancy of some glucocorticoid receptor within the paraventricular nucleus of the hypothalamus and pituitary is not necessarily sufficient to suppress stress-induced hypothalamic–pituitary–adrenal axis activity. **© 2006 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: HPA axis, glucocorticoid receptor, i.c.v. drug delivery, drug diffusion, corticosterone, negative feedback.

I.c.v. drug delivery is widely utilized to differentiate central versus peripheral drug effects. Briefly, i.c.v. delivery is accomplished by surgically implanting a cannula into the ventricular space such that injections can be made directly into the cerebrospinal fluid. It is assumed that diffusion throughout the cerebrospinal fluid (CSF) and surrounding tissue permits delivery to areas adjacent to the site of injection as well as to more distal brain regions; however, several researchers have reported rapid clearance from the ventricular system and hence very limited diffusion into brain (Aird, 1984; Crawley et al., 1991; de Lange et al., 1994; Ghersi-Egea et al., 1996).

A central focus of the research in our laboratory is stress neurobiology and regulation of hypothalamic–pituitary–adrenal (HPA) axis activity. A hallmark of HPA axis regulation is negative feedback inhibition of corticotropin releasing hormone and adrenocorticotropin hormone (ACTH) production and secretion by corticosterone (CORT) [for review of HPA physiology see (Dallman et al., 1987; Herman and Cullinan, 1997; de Kloet et al., 1998)].

In examining mechanisms of CORT negative feedback of the HPA axis, we performed experiments utilizing the glucocorticoid receptor (GR) agonist RU28362. Although a potent suppressor of stress-induced HPA activation when administered i.p. (Ginsberg et al., 2003), we were unable to observe an effect of RU28362 when acutely delivered i.c.v. One possible explanation for the observed lack of HPA suppression is poor drug delivery to essential sites for negative feedback.

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Abbreviations: ACTH, adrenocorticotropin hormone; ADX, adrenalectomy/adrenalectomizing; ANOVA, analysis of variance; CORT, corticosterone; CSF, cerebrospinal fluid; GR, glucocorticoid receptor; HBC, 2-hydroxypropyl-beta-cyclodextrin; HPA, hypothalamic–pituitary–adrenal; MDR, multiple drug resistance; PVN, paraventricular nucleus of the hypothalamus.

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Taking advantage of the dynamics of GR action, we were able to assess the extent to which RU28362 could diffuse away from the ventricular system following i.c.v. injection. When unbound by ligand, GR is found exclusively in the cytoplasm; when bound, GR translocates to the nuclear compartment within minutes (Raaka and Samuels, 1983; Munck and Holbrook, 1984; Madan and DeFranco, 1993; Sackey et al., 1996). By first adrenalectomizing (ADX) animals to remove endogenous glucocorticoids, we could then employ GR immunohistochemistry to clearly identify the extent of RU28362 diffusion away from the ventricular system. Nuclear GR immunostaining indicates areas of good drug penetration, whereas cytoplasmic immunostaining indicates areas of little or no RU28362 diffusion. Because of their relative GR enrichment and putative roles in HPA control, we chose for these studies the paraventricular nucleus of the hypothalamus (PVN) and dorsal hippocampus as two brain areas in which to quantify the degree of GR activation (McEwen, 1982; Dallman et al., 1992, 1987; Herman et al., 1989, 1992).

We propose that this methodology for tracking drug distribution has several advantages over other techniques such as microdialysis or administration of radiolabeled ligand (Aird, 1984; Crawley et al., 1991; de Lange et al., 1994; Ghersi-Egea et al., 1996). Utilizing GR immunohistochemistry permits excellent anatomical resolution and identifies ligand-activated receptors rather than the mere presence of the ligand. Thus, confounds such as the presence of ligand in adjacent capillaries or low non-functional ligand levels in extracellular space are eliminated.

We present here a series of studies examining the ability of RU28362 to occupy GR in the brain and pituitary following i.c.v. injection. The results indicate that RU28362 diffusion into brain following i.c.v. injection is very limited. We saw a similar limited diffusion of CORT after i.c.v. injection. Further, we saw evidence for the presence of RU28362 in the pituitary within 10 minutes after i.c.v. administration. Thus, it appears that poor diffusion into brain and rapid clearance of drug from the CSF may permit peripheral actions of RU28362 administered i.c.v., but likely preclude direct delivery to most brain targets.

EXPERIMENTAL PROCEDURES

Subjects

Male Sprague–Dawley Rats (Harlan Laboratories, Indianapolis, IN, USA) weighing between 225 and 290 g were used for all experiments. Rats were allowed at least 1 week acclimation before any surgical procedures. Throughout the studies, animals were housed two per polycarbonate tub with wood shavings and were given food (Purina Rat Chow; Ralston Purina, St. Louis, MO, USA) and tap water *ad libitum*. The colony room lights were regulated on a 12-h light/dark cycle, with lights on at 07:00 h. Procedures for ethical treatment of animals conformed to the guidelines found in the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996) and were approved by the University of Colorado Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

Experiment 1: dose response for RU28362 i.c.v.

Animals were implanted with guide cannula in the right lateral ventricle and allowed a minimum of 7 d recovery. The lateral ventricle injection site was chosen for both its proximity to forebrain areas as well as its position “upstream” from other CSF reservoirs. On the test day, animals received vehicle (n=6) [40% 2-hydroxypropyl-beta-cyclodextrin (HBC, H107; Research Biochemicals International, Natick, MA, USA) in saline], 0.005 μg RU28362 (n=7) (a gift from the former pharmaceutical company Roussel Uclaf, France), 0.1 μg RU28362 (n=7), or 1.0 μg RU28362 (n=6) in a total volume of 2 μl. Following injection, rats were returned to their home cages. After 1 h, subjects were placed in plastic restrainers [clear, ventilated, cylindrical Plexiglas tubes with adjustable length (15.5$\pm$2.5 cm length)] for 60 minutes. Blood samples were collected via tail nick at the beginning of restraint, 30 and 60 minutes later, and finally 1 h after the end of restraint for CORT radioimmunoassay. Following the functional challenge, animals received ADX surgery and were permitted 3 d recovery. On the second test day, animals (n=4) were administered either 2 μl vehicle or one of the three RU28362 doses from the functional test i.c.v. or 150 μg/kg RU28362 i.p. (40% HBC in saline, 0.3 ml total volume). The 150 μg/kg i.p. dose is six times the effective dose for HPA suppression and has been demonstrated to fully saturate available brain GR (Ginsberg et al., 2003). One hour later, animals were perfused with buffer and fixative and brains collected for immunohistochemistry.

Experiment 2: RU28362 versus CORT i.c.v.

Animals were implanted with guide cannula in the right lateral ventricle and allowed a minimum of 7 d recovery. On the test day, animals received vehicle (2% ethanol in saline), 1 μg RU28362, or 1 μg CORT (Steraloids Inc., Newport, RI, USA) in a total volume of 2 μl (n=4). Following injection, rats were returned to their home cages. After 1 h, subjects were placed in plastic restrainers for 30 min. Blood samples were collected via tail nick at the beginning and end of restraint for CORT radioimmunoassay. Following the functional challenge, animals received ADX surgery and were permitted 3 d recovery. On the second test day, animals were administered either 2 μl vehicle (n=3), 1 μg RU28362 (n=3), or 1 μg CORT i.c.v. (n=4); 150 μg/kg RU28362 (n=2), or 150 μg/kg CORT i.p. (n=2). One hour later, animals were perfused with buffer and fixative and brains collected for immunohistochemistry.

Experiments 3 and 4: time course for 1 μg RU28362 i.c.v. diffusion

The third experiment was a short time course in which animals were implanted with guide cannula in the right lateral ventricle, ADX, and allowed a minimum of 7 d recovery. On the test day, animals were administered 1 μg RU28362 or 2 μl vehicle (40% HBC in saline) i.c.v., or 150 μg/kg RU28362 i.p. Vehicle- (n=4) and i.p.- (n=3) treated animals were perfused 1 h after injection, while RU28362 i.c.v.-treated groups were perfused with buffer and fixative 10 (n=4), 30 (n=4), or 60 (n=4) minutes after injection. Brains and pituitaries were collected for immunohistochemistry.

In the fourth experiment, an extended time course, animals were implanted with guide cannula in the right lateral ventricle, ADX, and allowed a minimum of 7 d recovery. On the test day, animals were administered 1 μg RU28362 or 2 μl vehicle (2% ethanol in saline) i.c.v., or 150 μg/kg RU28362 i.p. Vehicle- (n=2) and i.p.- (n=2) treated animals were perfused 1 h after injection, while the i.c.v. drug groups were perfused with buffer and fixative 60 (n=4), 120 (n=4), or 180 (n=4) minutes after injection. Brains and pituitaries were collected for immunohistochemistry.

Surgical procedures

For both bilateral ADX and unilateral i.c.v. guide cannula surgeries, rats were fully anesthetized under ketamine (50 mg/kg) and

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