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EVIDENCE FOR A BIOACTIVE CLONIDINE-DISPLACING SUBSTANCE IN PERIPHERAL TISSUES AND SERUM

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Abstract—Clonidine-displacing substance (CDS) from brain is biologically active in the kidney and stomach and on platelets. To determine whether CDS is contained in these and other peripheral tissues, homogenates of fresh brain, eight other organs and serum from rat were ultrafiltered (<10,000 mol. wt only), dried and extracted with methanol. Evaluation by radioimmunoassay (RIA) using antibodies to *p*-aminoclonidine showed that adrenal gland and gastric fundus (GF) contained significantly greater amounts of CDS-like radioimmunoactivity than brain; intermediate-to-low activity was present in heart, small intestine, serum, kidney and liver; lung and skeletal muscle values were near-background. RIA-positive extracts elicited well-correlated contractile activity in a GF smooth muscle bioassay; contractions persisted in the presence of antagonists of various transmitters and modulators, but were abolished by low concentrations of the calcium channel blocker verapamil. Serum levels of CDS were profoundly reduced following removal of the adrenal glands. We conclude that a CDS-like substance is present not only in brain as previously reported, but also in peripheral organs and in the circulation.

Clonidine-displacing substance (CDS)‡ is a low molecular weight (<1000 Da), non-peptide substance found in brain that displays certain receptor binding properties similar to those of the synthetic agent clonidine [1, 2]. In the present study, we sought to determine whether CDS, also present in serum [3] and a neurally-derived cell line [4], is more widely—yet specifically—distributed in the body. Since the chemical structure of CDS is not known and therefore its presence not assayed directly, two methods of measurement were selected that are based on very different characteristics of the substance. The first was radioimmunoassay (RIA) since CDS cross-reacts with highly-specific polyclonal antibodies raised against a protein conjugate of *p*-aminoclonidine (PAC) [5–7]. The second assay was based on the ability of CDS to elicit contraction of the gastric fundus (GF) from the rat. Contraction of GF by CDS from brain has been shown to be dose dependent [8] and not attributable to other co-isolated low molecular weight substances that modify GF contractility, tested either by agonist cross-desensitization or antagonist blockade [9]. The presence of CDS in eight peripheral tissues from rat was evaluated by both assay methods, and only those tissues that demonstrated well-correlated activities from both were considered to contain a CDS-like substance. With this approach, results can be interpreted with far greater reliability than in studies in which a single assay is used.

A preliminary report related to this study [10] showed that the adrenal glands of the rat contained high levels of CDS-like radioimmunoactivity. Because of this finding, and the fact that CDS appears and acts within the circulation [3, 11], it is tempting to consider the role of CDS as a hormone. We therefore wished to establish whether the adrenal gland is a source of the CDS found in the circulation by assaying serum from rats before and after adrenalectomy.

METHODS

Isolation of CDS

Adult, male Sprague–Dawley rats (300–350 g) were decapitated, and blood was collected on ice. Whole brain and eight other tissues were removed and weighed; serum was prepared and pooled from the blood of several animals and kept at −70°. Methods for the rapid extraction of CDS from multiple tissue samples have been described [10]. Fresh tissues were coarsely minced and, in order to immediately denature large proteins, were homogenized in 3 vol. (v/w) of boiling, high-purity water (Burdick & Jackson) using a tissue homogenizer (Polytron, Brinkmann; setting 6, 2 × 30 sec). Homogenates were centrifuged at 100,000 *g* for 30 min at 4°. Supernatants and/or thawed serum samples were adjusted to 7% in trifluoroacetic acid and recentrifuged; supernatants from this step were then vacuum-dried at −15° in a Speed-Vac concentrator

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‡ Abbreviations: CDS, clonidine-displacing substance; RIA, radioimmunoassay; GF, gastric fundus; PAC, *p*-aminoclonidine; PGE_2, prostaglandin E_2; and VIP, vasoactive intestinal peptide.

equipped with a refrigerated circulator (Savant Instruments). Samples were reconstituted in 5–15 mL of ice-cold water, then centrifugally ultrafiltered (10,000 mol. wt cut-off; Centriprep 10, Amicon). The low molecular weight filtrates containing residual trifluoroacetic acid were adjusted to pH 6.8 to 7.8 using 0.5 to 1.0 M sodium hydroxide or, in some cases, 1.0 M ammonium hydroxide. Neutralized filtrates were then vacuum-dried overnight at −15° and extracted by bath-sonicating the residues with 30 vol. (v/dry wt) of ice-cold, high-purity methanol (Burdick & Jackson), followed by gravity filtration. The methanol-soluble fractions were dried and stored at −20°. Within 3 days of extraction and immediately prior to assay for CDS, individual samples were reconstituted in 0.8 to 1.0 mL of ice-cold, high-purity water, and then tested by RIA and bioassay (see below).

[^{3}H]PAC radioimmunoassay

Tissue samples were assessed by RIA in which the presence of a CDS-like substance was established by displacement of [^{3}H]PAC from anti-PAC antibody binding sites. The use of polyclonal antibodies produced in rabbit (anti-PAC_2) in a rapid-filtration assay has been described [5–7, 10]. Samples containing CDS (100 μL) were preincubated with an aliquot of ammonium sulfate-precipitated anti-PAC_2 immunoglobulins (1:10,000 final dilution) in 50 mM Tris–HCl, pH 7.4, at 25° for 15 min. [^{3}H]PAC (1 nM) was then added and incubated (final volume, 0.5 mL) for 15 min, followed by 1 hr on ice. The reaction was terminated with addition of bovine γ-globulin and polyethylene glycol 8000, and samples were vacuum filtered over Whatman GF/B filters using a modified cell harvester. Filters were washed, and then counted at 45–50% efficiency. Radioimmunoactivity was calculated as percent inhibition of the total specific [^{3}H]PAC binding to anti-PAC_2 using 1 nM radioligand and 10 μM unlabeled PAC to define nonspecific binding.

To report tissue levels, extract displacement curves were analyzed by linear regression of Hill plots using LIGAND, a program for multivariate least squares analysis [see Ref. 7]. The amount of extract yielding 50% inhibition was determined from the Hill plots and defined as one RIA-Unit of CDS-like radioimmunoactivity. Pseudo-Hill coefficients (n_H) were reported as an average slope obtained from Hill plots for the individual tissue extracts assayed.

Concentration-related inhibition by unlabeled PAC (concentration range, $10^{-9.5}$ to 10^{-7} M) was used as a standard control in all experiments. In the present study, PAC standard IC_{50} values averaged 1.8 nM, with an error of 0.2 nM (N = 30).

Bioassay: Contraction of gastric fundus smooth muscle from rat

Contraction of rat GF smooth muscle, as outlined previously [8, 9], was used as a bioassay for CDS. Rats were decapitated and the stomachs removed and placed in ice-cold, oxygenated Krebs–Henseleit medium (in mM: NaCl, 120; KCl, 4.7; $MgSO_4 \cdot 7\,H_2O$, 1.2; KH_2PO_4, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; dextrose, 10.0; $NaHCO_3$, 25.0). The stomach was dissected along the greater curvature, the contents were extruded and the tissue was rinsed in fresh medium. The fundus was isolated from the body of the stomach, cleaned, and divided at the midpoint into two uniform strips. Four strips total were suspended from force transducers by silk sutures and arranged in a cascade [12]. The strips were superfused with fresh medium at 10 mL/min; an initial tension of 1.0 g was applied and the system allowed to equilibrate for 30–60 min.

The contractile responsivity of the GF strips was then calibrated using 200 pmol of prostaglandin E_2 (PGE_2) as a standard for eliciting maximal contraction [8]. (Sample contractions were expressed as a fraction of this response, as indicated below.) Reconstituted extracts of the tissues to be tested for CDS-like contraction were applied individually to the cascade in a bolus at a series of dilutions and the results were recorded on a polygraph. All samples were kept on ice during the bioassay.

To rule out interfering activities of agents other than CDS in the tissue extracts (e.g. acetylcholine, adenosine, histamine, norepinephrine, serotonin, and opiates and other peptides), extracts were then tested in the presence of a solution [9] containing, at a final concentration in μM: atropine (1.0), chlorpheniramine (10), idazoxan (1.0), indomethacin (10), metiamide (10), naloxone (1.0), phenoxybenzamine (10), propranolol (1.0), tetrodotoxin (0.1), theophylline (1.0), Sar^1-Ala^1-angiotensin II (0.03), des-Arg^9-Leu^8-bradykinin (1.0), and Pen^1-Val^4-Arg^8-vasopressin (0.5). The solution was infused over the cascade of fundic strips at a constant rate of 0.1 mL/min; then tissue extract was added as a bolus and the results were recorded. The effect of the calcium channel blocker verapamil (10 nM final concentration) was tested in a separate trial by adding verapamil to the infused antagonist solution and re-introducing a bolus of each extract.

Contractile activity, with or without antagonists, was calculated as the index: peak tension (g)/[standard peak tension$_{200\,pmol\,PGE_2}$(g) × g wet wt]. A CDS-like contractile response (g^{-1}) was concentration dependent, persisted in the presence of the antagonists tested, and was abolished by low nanomolar concentrations of verapamil indicating a high dependence on calcium channels [9].

Specific responses for 0.01 to 10 nmol levels of several other neuroactive agents—for which specific antagonists were not included in the superfusion medium—were also compared with CDS-like contractions. Cross-desensitization of these agents with CDS was tested after multiple bolus applications had been applied [9]: dopamine (0.1 mM stock with 0.1% ascorbic acid), corticotropin releasing factor (1.0 nM), galanin (100 nM), neuropeptide Y (0.1 mM), thyrotropin releasing hormone (10 mM), and vasoactive intestinal peptide (VIP, 100 nM). No cross-desensitization with CDS-like contractile responses, regardless of the tissue extract being tested, was observed.

Adrenalectomy

Rats (300–350 g) were weighed and anesthetized (2% Fluothane in O_2 by nasal inspiration), and a transverse, dorsal incision was made. Adrenal glands were dissected free of surrounding tissues, tied off,

and removed intact. In the case of sham-operated controls, adrenal glands were exposed but not excised. All wounds were closed, and the animals returned to their cages. Maximum time of anesthesia was 30 min; all animals regained consciousness within 5–7 min and tolerated the procedure without complication. Recovering rats were sustained on a diet of Purina rat chow and 0.9% saline *ad lib.* Decapitation followed 72 hr after surgery. Blood was collected into plastic centrifuge tubes on ice; serum was prepared, pooled from five adrenalectomized or control rats, and stored at −70° until just prior to processing and methanol extraction of CDS. Content of CDS-like radioimmunoactivity and bioactivity were determined for both groups as described above.

Materials

The radioligand [^{3}H]PAC (48.5 Ci/mmol) was obtained from New England Nuclear (Boston, MA); it was stored at −20° in ethanol and diluted in high-purity water just prior to RIA. Neuropeptide Y, thyrotropin releasing hormone, and des-Arg^9-Leu^8-bradykinin were obtained from Peninsula laboratories (Belmont, CA); propranolol was from Research Biochemicals International (Natick, MA). Idazoxan was a gift from Reckitt–Colman (Kingston-upon-Hull, U.K.) All other compounds were purchased from the Sigma Chemical Co. (St. Louis, MO). In general, for GF bioassay, test compounds were prepared in 0.9% saline.

RESULTS

Brain: CDS radioimmunoactivity and GF contractile activity

Prior to screening peripheral tissues from rat, we wished to confirm the presence of CDS in rat brain. To do this, we compared the characteristics of rat brain extracts to those previously described for bovine brain, that is, binding displacement in an RIA using specific anti-PAC antibodies [7], and GF smooth muscle bioactivity [8, 9].

Like bovine brain [7], rat brain contained CDS-like radioimmunoactivity (Fig. 1A). The displacement of the total specific binding of [^{3}H]-PAC to anti-PAC$_2$ antibodies by rat brain extract was concentration dependent and, as previously defined [5–7], the amount resulting in 50% inhibition was taken as one RIA-Unit of CDS-like activity.

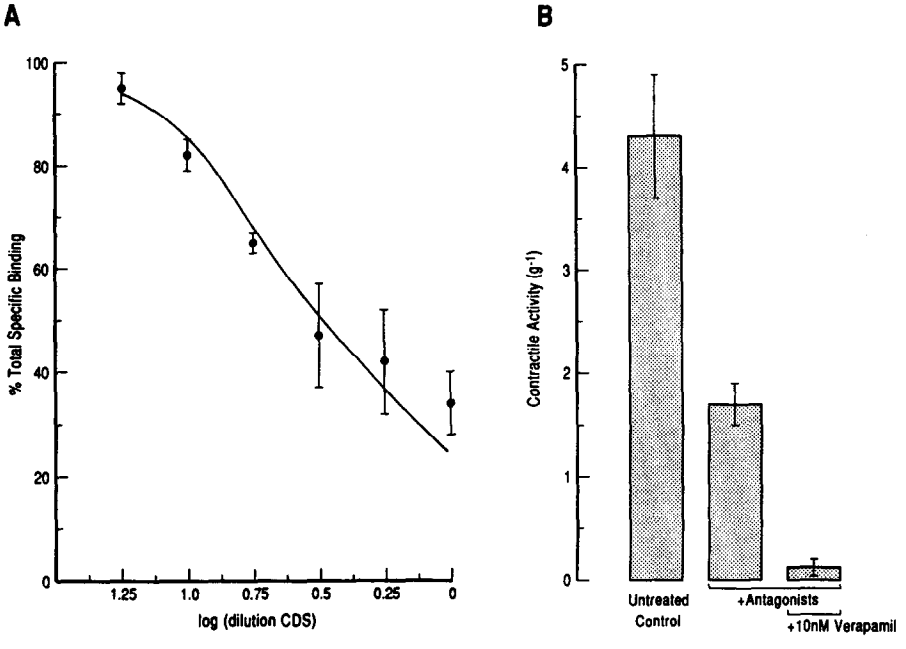

Fig. 1. (A) Inhibition of binding of [^{3}H]PAC to anti-PAC$_2$ antibodies by rat brain CDS. Preparation of extracts, precipitation of polyclonal antibodies from whole serum using 50% ammonium sulfate, and incubation of increasing amounts of brain extract with anti-PAC$_2$ antibodies and [^{3}H]PAC as radioligand were all carried out as detailed in Methods. The total specific [^{3}H]PAC binding remaining in the presence of each concentration of brain extract was calculated as percentage of the total binding minus the nonspecific binding defined by 20 μM unlabeled PAC. Data represent the means ± SEM of triplicate values from 4 experiments. (B) Effect of rat brain extract on gastric fundus (GF): characteristics of CDS-like contractile activity. Extract was applied as a bolus to control (untreated) GF strips, eliciting the response shown. Strips were then treated via constant infusion (0.1 mL/min) with the solution of antagonists/inhibitors listed in Methods, and the extract was re-applied. Extract was applied a third time after adding 10 nM verapamil to the superfusion medium. Responsiveness of the GF strips was tested throughout the experiment by applying PGE_2 standard. Contractile activity is expressed as an index: peak tension (g)/(standard peak tension elicited by 200 pmol PGE_2(g) × g wet wt tissue extracted). Data are means ± SEM, N = 8.

Table 1. Distribution of CDS-like radioimmunoactivity in peripheral tissues of the rat

Tissue	CDS-like radioimmunoactivity (RIA-Units/g wet wt)	Pseudo-Hill coefficient (n_H)	N
Brain	9 ± 2	1.3 ± 0.2	10
Adrenal gland	114 ± 16*	1.0 ± 0.3	8
Gastric fundus	64 ± 17†	1.3 ± 0.2	9
Heart	9 ± 3	0.92 ± 0.08	8
Small intestine	3 ± 1	1.3 ± 0.3	6
Kidney	1.8 ± 0.4	0.8 ± 0.1	10
Liver	1.5 ± 0.3	1.2 ± 0.2	10
Lung	0.4 ± 0.2	0.27 ± 0.04‡	4
Skeletal muscle	0.5 ± 0.5	0.4 ± 0.1‡	5
Serum	7 ± 2§	1.2 ± 0.2	11

Values are means ± SEM of N experiments.
* Significantly greater than all other tissues ($P < 0.01$, ANOVA with Newman–Keuls test).
† Significantly less than adrenal gland ($P < 0.01$, ANOVA with Newman–Keuls test).
‡ Significantly less than n_H for all other tissues ($P < 0.05$).
§ RIA-Units/mL.

Rat brain contained 9 ± 2 RIA-Units/g wet wt (N = 10) (Table 1), compared to an estimated 0.5 RIA-Units/g of bovine brain [unpublished; see Ref. 7]. Furthermore, rat brain CDS, like bovine [7], appeared to interact with a single class of [^{3}H]PAC recognition sites on anti-PAC_2 antibodies since the pseudo-Hill coefficient of the inhibition curve was 1.3 ± 0.2 ($r > 0.9$; N = 10) (Table 1).

CDS extracted from rat brain elicited a concentration-dependent contraction of GF smooth muscle exactly comparable to that observed with extracts of bovine brain [see response trace in Ref. 8]. Extract was applied to control (untreated) GF strips (N = 8) and a contractile response of the magnitude indicated in Fig. 1B was obtained (contractile activity is reported as defined in Methods). After treating the strips with a solution of adrenergic, cholinergic, monoaminergic and peptidergic antagonists, as well as indomethacin to block prostaglandin synthesis and tetrodotoxin to block sodium channels, brain samples were re-applied. CDS-like contraction of GF persisted (40% of control) after treatment with antagonists (Fig. 1B). Addition of a low concentration (10 nM) of the calcium channel blocker verapamil resulted in complete abolition (not significantly different from baseline) of the CDS-like contraction. This result directly mirrored that observed with bovine brain tissue extracts [9]. Thus, there appeared to be a CDS-like substance in rat brain extracts, i.e. having assay properties identical to those defined in bovine brain, even though the current preparation of rat extract was a modification of the original procedure [7, 8].

Peripheral tissues

CDS-like radioimmunoactivity. To investigate whether a substance similar to the CDS in brain was contained in peripheral tissues, eight organs and whole serum from rat were extracted and assayed for CDS-like radioimmunoactivity [5–7]. Results from the [^{3}H]PAC RIA are shown in Table 1. Values are expressed in RIA-Units (see Methods) per gram wet weight of tissue or per mL of serum.

Adrenal gland and gastric fundus contained levels of CDS-like radioimmunoactivity per gram of tissue that were 12- and 6-fold greater than brain, respectively. These tissues also exhibited significantly greater activity than the others assayed ($P < 0.01$, ANOVA with Newman–Keuls): heart > small intestine > kidney, liver. Background levels (not significantly different from the lower limit of the assay) were observed in lung and skeletal muscle. CDS-like radioimmunoactivity was also detected in serum (note RIA-Units are expressed per mL).

As with brain extracts, the interaction of anti-PAC_2 antiserum with extracts of adrenal gland, gastric fundus, heart, small intestine, kidney, liver and serum appeared to occur via a single class of recognition sites under the standard assay conditions used. Pseudo-Hill coefficients were near unity as shown in Table 1. It is noteworthy that coefficients for lung and skeletal muscle were less than unity in contrast to the other tissues (N = 4–5; $P < 0.05$).

Bioactivity. Tissue extracts that contained CDS-like radioimmunoactivity were also evaluated by bioassay.

Various extracts elicited contraction of rat GF; however, three classes of responses could be described based on the sensitivity to antagonists, or to verapamil. In the first, responses were characteristic of those defined by brain CDS; that is, they were consistent, concentration-dependent contractions of GF that persisted in the presence of antagonists (Table 2) and were inhibited completely (not significantly different from baseline) by 10 nM verapamil (Fig. 2). Using these criteria, extracts of gastric fundus, heart, kidney, and serum were considered "fully bioactive" and, because they also exhibited significant radioimmunoactivity, were designated as containing a CDS-like substance.

The second class of responses was found with small intestine and liver. These contractions were concentration dependent and resistant to blockade

Table 2. Effect of antagonist treatment on CDS-like contraction of rat gastric fundus

Tissue	Contractile activity* Control GF (−antagonists)	Treated GF (+antagonists)	% Control
Brain	4.3 ± 0.6	1.7 ± 0.2†	40
Gastric fundus	12 ± 1	7.0 ± 0.9†	58
Heart	0.8 ± 0.1	11.1 ± 0.5	140
Small intestine	3.7 ± 0.6	1.5 ± 0.4†	41
Kidney	1.8 ± 0.3	1.9 ± 0.6	110
Liver	1.9 ± 0.3	1.0 ± 0.2†	53
Lung	2.1 ± 0.3	0.8 ± 0.3†	38
Skeletal muscle	1.6 ± 0.3	1.6 ± 0.3	100
Serum	1.7 ± 0.3‡	0.5 ± 0.1†‡	29

* Values represent the index: peak tension (g)/[standard PGE_2 peak tension (g) × wet wt (g)]; mean ± SEM, N = 6–21.

† Significantly different compared to control (−antagonists) contractile response ($P < 0.05$, unpaired t-test).

‡ Expressed per mL.

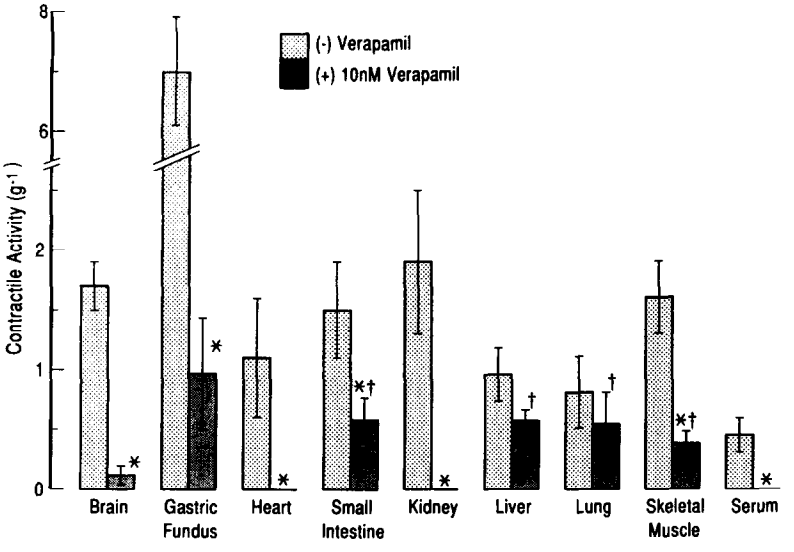

Fig. 2. Antagonist-resistant contraction of GF smooth muscle by extracts of rat peripheral tissues, before and after verapamil treatment. Contractile activity represents an index normalized for gram wet weight of tissue in each extract (mean ± SEM, N = 5–8) as indicated in Methods. All data were obtained following antagonist/inhibitor treatment of GF strips. Significant differences were determined from unpaired t-tests of individual values. Key: * bioactivity significantly less than control (without verapamil) ($P < 0.05$, ANOVA with Newman–Keuls); and † verapamil-insensitive bioactivity significantly greater than background ($P < 0.05$, ANOVA with Newman–Keuls).

by antagonists (Table 2). Yet, responses were not abolished completely by low concentrations of verapamil (Fig. 2), perhaps because of co-isolation of masking-levels of contractile peptides that are insensitive to such low concentrations of verapamil. In this class, 60 and 40% of the responses to small intestine and liver, respectively, were CDS-like.

In the third class, lung and skeletal muscle extracts contracted GF smooth muscle (Table 2). However, the characteristics of the responses were not CDS-like, differing in latency and duration (data not shown) and, in the case of lung extracts, lacking sensitivity to 10 nM verapamil (Fig. 2). The rapid spike contractions of skeletal muscle extracts were identical to those observed with potassium chloride and, in fact, potassium levels in these extracts were 40 mM by flame photometry (N = 2). Thus, lung and skeletal muscle appeared devoid of CDS-like activity.

Extracts of adrenal gland could not be characterized as belonging to any of the three classes of bioactivity above. Despite significantly greater CDS-like

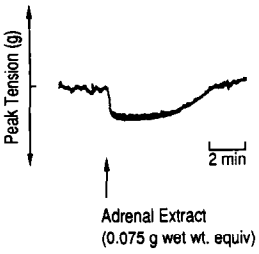

Fig. 3. Relaxation of GF elicited by rat adrenal gland extract. The effect of adrenal extracts was determined in the presence of an antagonist/inhibitor solution containing 10 nM verapamil. Relaxation was observed, which was immediate in onset (bolus application of extract indicated by arrow), concentration dependent (not shown), and profound and long-lasting (>1 g fall in peak tension per 75 mg equivalents of adrenal tissue, lasting approx. 4 min). The trace shown is representative of trials from 6 GF strips.

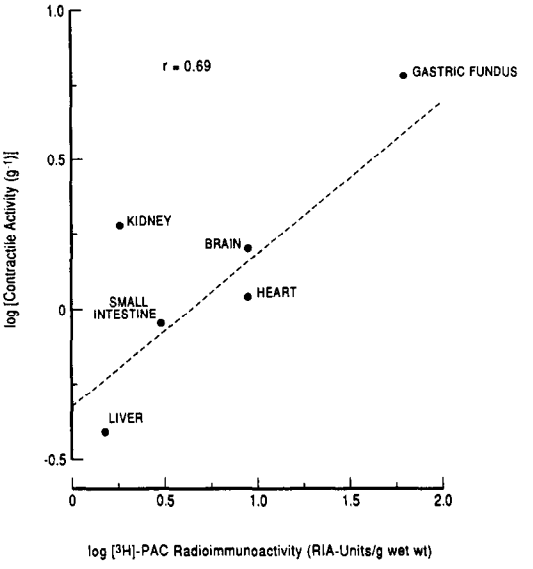

Fig. 4. Log–log relationship of CDS-like (verapamil-sensitive, antagonist-resistant) contractile activity to CDS-like radioimmunoactivity for six peripheral tissue extracts. Data represent mean values from 6–10 RIA experiments for the different tissues indicated, performed in triplicate at three dilutions, and 6–8 GF strips tested in at least two trials.

radioimmunoactivity than brain (see Table 1), adrenal extracts consistently elicited relaxation and not contraction of GF smooth muscle (Fig. 3). The relaxation was (a) concentration dependent, (b) immediate in onset, (c) profound and long-lasting (>1 g fall in peak tension per 8 RIA-Units with a duration of 4 min), and (d) insensitive to pretreatment of GF with various antagonists or verapamil. The response persisted during α- and β-adrenergic blockade (phentolamine, 1.0 μM, and propranolol, 10 μM, respectively), indicating that it was not due to co-extraction of catecholamines. Several adrenal peptides for which antagonists were not included in the pretreatment superfusion medium (cholecystokinin octapeptide, 300 pmol; corticotropin releasing factor, 1 nmol; neuropeptide Y, 10 nmol; and thyrotropin releasing hormone, 10 nmol) also had no effect on GF tension. In contrast, VIP elicited a concentration-dependent relaxation that was identical in onset and duration to that of adrenal extracts (data not shown). VIP has been identified in rat adrenal gland [13, 14] and shown by others to relax gastric smooth muscle [15]. VIP may thus mask CDS-like contractile activity of partially-purified adrenal extracts.

Correlation of two independent assays

The correlation of the two methods of assay for CDS was evaluated by comparing the CDS-like radioimmunoactivity (RIA-Units) with the antagonist-resistant, verapamil-sensitive (CDS-like) component of the GF contraction for the six tissues that gave positive responses by both methods; a log-log scale was used to equally weight the broad range of activities observed (Fig. 4). Responses to the tissue extracts in the two assays were positively correlated ($r = 0.69$). In fact, the correlation between assays increased ($r = 0.99$) when the data for kidney (bioactivity ≫ radioimmunoactivity) was not included in the regression analysis, and remained high ($r = 0.92$) when GF values (much greater than other tissues) were not considered. Thus, strong positive correlation between two independent assays for five peripheral tissues establishes CDS as a substance that, in addition to its clonidine-displacing activity, exhibits radioimmunoactivity and elicits a specific contraction of GF smooth muscle.

CDS in the circulation

If the abundant CDS-like radioimmunoactivity in rat adrenal gland extracts is, in fact, due to CDS, the adrenals may be a source of the CDS measured in serum. To test this hypothesis, we prepared extracts of serum pooled from five adrenalectomized rats and age-matched controls for RIA. The CDS-like radioimmunoactivity in serum of adrenalectomized rats (N = 5 groups) was significantly less ($P < 0.001$) than that of controls (0.2 ± 0.2 and 11 ± 2 RIA-Units/mL, respectively). Moreover, the pseudo-Hill slopes of the dilution curves for serum extracts from the adrenalectomized groups were variable and less than unity (0.4 ± 0.5), whereas the interaction of control extracts with anti-PAC_2 antibodies appeared to be at a single recognition site (pseudo-Hill slope, 1.0 ± 0.1). Thus, a CDS-like substance in serum may be released from the adrenal glands.

DISCUSSION

This report is the first to demonstrate the tissue-specific distribution of an endogenous CDS-like substance, confirmed by correlated responses in two assay systems. The specificities of the assays depend upon entirely different kinds of interactions with CDS—cross-reactive binding to anti-*p*-amino-clonidine antibody recognition sites (RIA) and

activation of sites on or within smooth muscle cells (GF contraction bioassay). The measure of these distinct, yet correlated, activities provides strong evidence that CDS or a CDS-like substance is present in peripheral organs of the rat: high concentrations in GF and moderate-to-low levels in serum, heart, small intestine and liver. Kidney extracts, as well as those of adrenal gland, require examination after further purification of the active substance(s) since their bioassays suggest the presence of other substances that elicit non-CDS-like contraction, and profound relaxation, respectively.

From the findings presented, questions concerning the biological role(s) of a CDS-like substance in the brain and the periphery arise. For some of the tissues assayed here, and several others, functional studies have already been initiated. For example, (a) CDS isolated from bovine brain is reported to have profound effects on arterial pressure when applied to the rostral ventrolateral medulla, a highly-integrative cardiovascular area of the brainstem [2, 16]. Thus, an endogenous substance with properties related to the synthetic antihypertensive clonidine appears to participate in central mechanisms regulating blood pressure. (b) In GF, CDS (isolated from brain or peripheral organs) has a functional activity described here and elsewhere [8, 9] that is relevant to gastric motility. (c) CDS has a biochemical effect on the Na^+/H^+ exchanger in kidney and, therefore, possibly on Na^+ excretion mechanisms [17, 18]. (d) In the blood, CDS has been observed to facilitate human platelet aggregation [11] and is higher in concentration in the serum of patients with pregnancy-induced hypertension relative to normotensive subjects [3]. Therefore, circulating and (or) intrinsic stores of CDS in these tissues may play a significant role in specific central medullary, gastric and renal functions. In addition, a biological role for authentic CDS in adrenal gland is suggested by our finding that CDS elicits a large release of catecholamines from chromaffin cells [19]. Biological functions in heart, small intestine and liver—reported here to also contain a CDS-like substance—have not yet been examined.

In an increasing number of tissues, a novel receptor type has been reported that (a) preferentially binds imidazol(in)e-containing compounds, (b) has been identified using either [^{3}H]clonidine or [^{3}H]PAC, or the imidazoline [^{3}H]idazoxan as radioligand, and (c) is termed either imidazol(in)e receptors (IR) or imidazoline-guanidinium-receptive sites (IGRS) [see Refs. 20 and 21 for review]. Receptors labeled by [^{3}H]PAC have been localized in brain tissue [2, 20–23] and in kidney [24]. Using [^{3}H]idazoxan as radioligand, IR/IGRS have been demonstrated in brain, kidney, urethra [reviewed in Refs. 20 and 21], liver [25, 26] and human fat cells [27]. IR/IGRS may represent the receptive site(s) of action for CDS [2, 17, 22]. CDS has been shown directly to interact with IR defined by [^{3}H]PAC in brain [2, 22] and kidney [24], and defined by [^{3}H]idazoxan in kidney [17], adrenal chromaffin cells [19], and in liver [25]. It is conceivable that the PAC (clonidine) and idazoxan receptor sites represent pharmacologically-selective subtypes of IRs that are differentially distributed, both in the brain and in a number of peripheral tissues [28], and that mediate the effects of CDS in these tissues.

One exception may be the contraction of GF used here as a specific bioassay for CDS. In this system, the mechanism(s) mediating contractile responses is thus far unknown. Since idazoxan was included in the antagonist solution—originally as an α_2-adrenergic blocker, yet subsequently found to bind with high affinity to the PAC as well as the idazoxan IR subtype—it is unlikely that the specific effects of CDS are transmitted via either of these apparent receptor subtypes. Though it is tempting to postulate yet a third IR subtype, further study is necessary for a reliable understanding of the functional mechanism(s) of CDS in GF.

The findings reported here represent the first indication that CDS is a molecular messenger that may be either accumulated and stored, or synthesized locally in a variety of peripheral tissues. Furthermore, the fact that removal of the adrenal glands results in a profound and significant decrease in serum CDS-like activity is provocative in that CDS may also act as a circulating hormone with far-reaching responses in multiple target organs. Whether the substance is released into the circulation from the adrenal cortex or the medulla remains to be established. Additional experiments are currently underway.

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