Molecular Neurobiology

ISSN0893-7648/92/5/2-3-4/251–273/$4.60

Central Nervous System Binding Sites for Calcitonin and Calcitonin Gene-Related Peptide

Patrick M. Sexton

St. Vincent's Institute of Medical Research, 41 Victoria Parade, Fitzroy, 3065, Victoria, Australia

Contents

Abstract

Alternative splicing of the primary RNA transcript of the calcitonin gene leads to the generation of two distinct peptides, calcitonin (CT) and calcitonin gene-related peptide (CGRP). These peptides share only limited sequence homology and generally subserve different biological functions through their own distinct binding sites, which differ in specificity and distribution. Additionally, a binding site with high-affinity binding for both peptides that has a restricted pattern of distribution has been identified. The present article reviews the biochemical and morphological characteristics of central CT and CGRP binding sites.

Index Entries: Calcitonin; calcitonin gene-related peptide; receptor; binding site; nervous system; autoradiography.

Introduction

Calcitonin and CGRP

The 32 amino acid peptide hormone calcitonin (CT) is secreted from the thyroid and acts to influence calcium homeostasis, primarily through inhibition of osteoclastic bone resorption (Nicholson et al., 1986; Martin et al., 1988), with a lesser action at receptors in the kidney to increase excretion of calcium and magnesium (Elalouf et al., 1986; Sexton et al., 1987). In the early 1980s, studies on the expression of CT in serially transplanted medullary thyroid carcinoma led to the discovery of a second species of messenger RNA from the calcitonin gene, termed calcitonin gene-related peptide (CGRP) mRNA (Amara et al., 1982). Production of CGRP mRNA arises from alternative processing of the primary RNA transcript with selection of different exons by selective use of alternative polyadenylation sites (Amara et al., 1984). This yields either mature CT mRNA or CGRP mRNA, which have common 5' coding regions, but different 3' regions (Fig. 1). Mature CT peptide contains 32 amino acids and is expressed primarily in the thyroid gland, whereas the 37 amino acid mature CGRP peptide is the predominant gene product in neuronal tissue (Rosenfeld et al., 1983). A gene encoding a second form of CGRP, termed β-CGRP, has also been identified in rats (Amara et al., 1985) and humans (Steenbergh et al., 1985). In humans, this peptide differs from α-CGRP in three residues and by only one residue in the rat (Fig. 2). CT and CGRP have now been isolated and sequenced from many species (Figs. 2 and 3) with the high degree of homology for CGRPs suggestive of an important and conserved physiological role during evolution (Lips et al., 1988). CT and CGRP share only limited amino acid sequence homology (Figs. 2 and 3), and appear to subserve different biological functions. Peripherally, CGRP has a diverse range of effects, including relaxation of vascular and other smooth muscle, and decreases in gastric acid secretion, as well as direct stimulation of rate and force of contraction in the heart (*see* Zaidi et al., 1990).

Central Binding Sites for CT and CGRP

We and others have mapped the distribution of binding sites for CT (Henke et al., 1983; Olgiati et al., 1983; Guidobono et al., 1987) and CGRP (Skofitsch and Jacobowitz, 1985a; Seifert et al., 1985, Henke et al., 1985; Sexton et al., 1986). Based on their distribution and specificity, at least three types of binding sites can be distinguished:

1. Conventional CT binding sites, defined using ^{125}I-salmon CT as radioligand, which show specificity for CT-related peptides, but not CGRP, and have a characteristic morphological pattern of distribution;
2. Conventional CGRP binding sites, defined using ^{125}I-CGRP (owing to the high degree of homology between CGRPs, the species of CGRP used as radioligand has little bearing on the results: *see* CGRP Binding Sites for discrepancies), show specificity for CGRP peptides, but not CT, and have a characteristic distribution distinct from that of CT binding sites; and
3. CT-sensitive CGRP binding sites, identified in rat brain using ^{125}I-rat CGRP as the radioligand, which have high affinity for both salmon CT and rat CGRP, and a restricted pattern of distribution (Sexton et al., 1988; Dennis et al., 1991).

There is, additionally, emerging evidence for further heterogeneity of the binding sites for CT and CGRP displaying conventional specificity for CT and CGRP peptides (*see below*), although the significance of these studies for central CT and CGRP receptors is unclear.

CT Binding Sites

Distribution

High-affinity binding sites for CT are present in homogenates of both rat (Rizzo and Goltzman, 1981; Fischer et al., 1981a; Nakamuta et al., 1981) and human (Fischer et al., 1981b) brain. Highest levels of binding occur in the hypothalamus, followed by brainstem, with intermediate binding in the striatum and midbrain thalamus, whereas

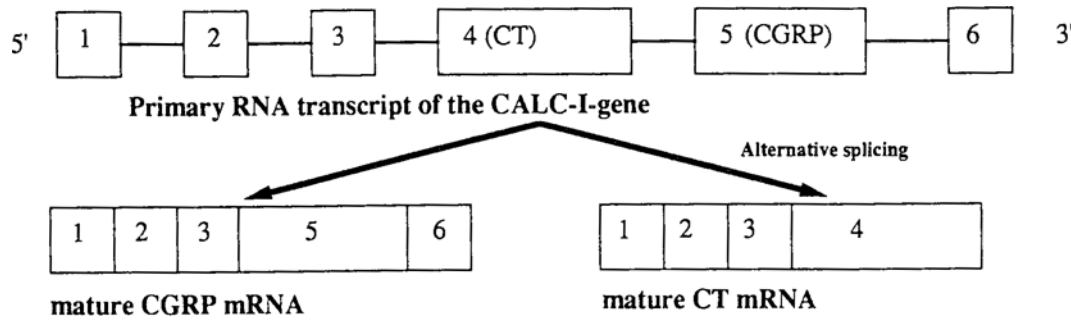


Fig. 1. Schematic diagram of alternative splicing of the primary RNA transcript of the CALC-I gene.

Peptide	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
hCGRPα	A	C	D	T	A	T	C	V	T	H	R	L	A	G	L	L	S	R	S
hCGRPβ	A	C	N	T	A	T	C	V	T	H	R	L	A	G	L	L	S	R	S
rCGRPα	S	C	N	T	A	T	C	V	T	H	R	L	A	G	L	L	S	R	S
rCGRPβ	S	C	N	T	A	T	C	V	T	H	R	L	A	G	L	L	S	R	S
cCGRP	A	C	N	T	A	T	C	V	T	H	R	L	A	D	F	L	S	R	S
pCGRP	S	C	N	T	A	T	C	V	T	H	R	L	A	G	L	L	S	R	S
rbCGRP	G	C	N	T	A	T	C	V	T	H	R	L	A	G	L	L	S	R	S
sCT	C	S	N	L	S	T	C	V	L	G	K	L	S	Q	E	L	H	K	L

	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
hCGRPα	G	G	V	V	K	N	N	F	V	P	T	N	V	G	S	K	A	F-NH$_2$
hCGRPβ	G	G	M	V	K	S	N	F	V	P	T	N	V	G	S	K	A	F
rCGRPα	G	G	V	V	K	D	N	F	V	P	T	N	V	G	S	E	A	F
rCGRPβ	G	G	V	V	K	D	N	F	V	P	T	N	V	G	S	K	A	F
cCGRP	G	G	V	G	K	N	N	F	V	P	T	N	V	G	S	K	A	F
pCGRP	G	G	M	V	K	S	N	F	V	P	T	N	D	G	S	E	A	F
rbCGRP	G	G	M	V	K	S	N	F	V	P	T	N	V	G	S	E	A	F
sCT	Q	T	Y	P	R	T	N	T	G	S	G	T	P-NH$_2$					

Fig. 2. Amino acid sequences of salmon CT and CGRP from different species. Homologous residues are blocked. Abbreviations are: h, human; r, rat; c, chicken; p, porcine; rb, rabbit; s, salmon.

negligible binding is found in the cortex, hippocampus, cerebellum, and spinal cord (Fischer et al., 1981a,b). Autoradiographic mapping of rat CT binding sites revealed high densities associated with parts of the ventral striatum, organum vasculosum of the lamina terminalis (OVLT), subfornical organ, median preoptic nucleus and medial preoptic area, periventricular and paraventricular hypothalamic nuclei, dorsal hypothalamic area, tuber cinereum, retrochiasmatic area, arcuate nucleus, and median eminence. High-density binding also occurred in the medial and central nuclei of the amygdala, substantia nigra pars compacta, periaqueductal gray, pontine and medullary reticular formation, most of the midline raphe nuclei, parabrachial nuclei, locus cerulus, nucleus of the solitary tract (NTS), and area postrema (Fig. 4, *see* pp. 258,259; Olgiati et al., 1983, Henke et al., 1983).

Comparison of binding site distribution across species demonstrates that regions of high-density binding including hypothalamus, amygdala,

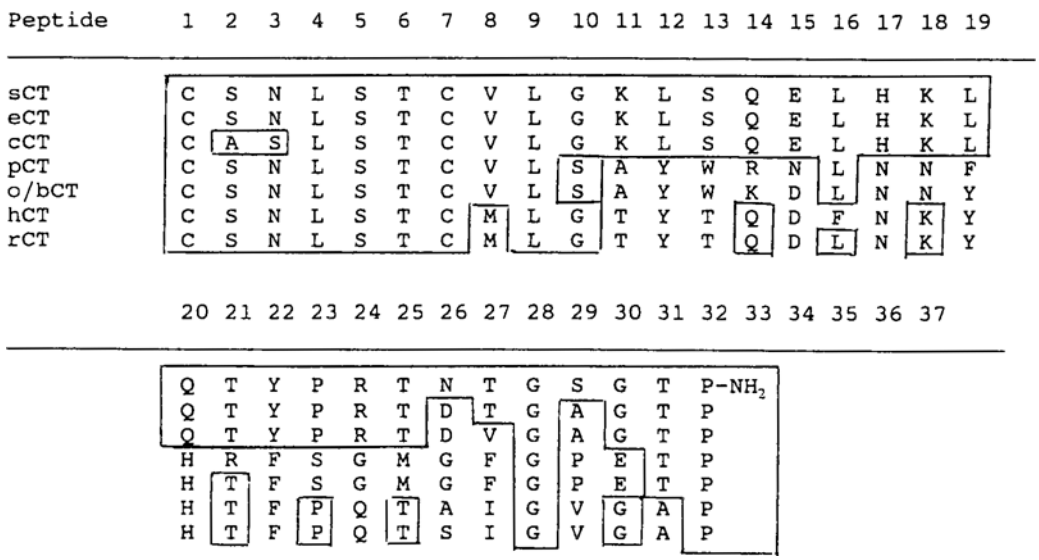


Fig. 3. Amino acid sequences of calcitonins from different species. Residues homologous to salmon CT are blocked. Abbreviations are: s, salmon; e, eel; c, chicken; p, porcine; o, ovine; b, bovine; h, human; r, rat.

and caudolateral putamen are conserved in sheep (Sexton et al., 1990,1991), rat (Fig. 4; Olgiati et al., 1983; Henke et al., 1983), cat (Guidobono et al., 1987), and human (Sagar et al., 1984; P. M. Sexton, unpublished data). The distribution of binding sites in cat and sheep, however, is more extensive than in rat/human, with binding present in the hippocampus and much of the thalamus. Accordingly, central CT binding sites are likely to subserve a subset of similar functions in all species, whereas additional actions may occur in species, such as cat and sheep.

Properties

Analysis of binding specificity for CT binding sites from rat, human, and sheep brain membranes (Fischer et al., 1981a,b; Rizzo and Goltzman, 1981; Nakamuta et al., 1981; Sexton et al., 1991) reveals a rank order of potency, with salmon CT > porcine CT > human CT, equivalent to functional CT receptors in other systems, including osteoclasts (Nicholson et al., 1986) and kidney (Sexton et al., 1987; Goltzman and Mitchell, 1985). CGRP was at least 500-fold less potent than salmon CT in inhibiting binding to brain membranes (Goltzman and Mitchell 1985; Sexton et al., 1991).

Recent studies have indicated heterogeneity in CT binding sites, including those of the CNS (Nakamuta et al., 1990; Twery et al., 1988). Based on the relative binding affinity of analogs of salmon CT, with either poor or strong helical-forming capacity, Nakamuta et al. (1990) described two binding site subtypes. These sites were denoted CT-linear and CT-helical with peripheral hypocalcemic potency paralleled by relative potencies of analogs at the "linear" binding subtype. The significance of these findings is unclear.

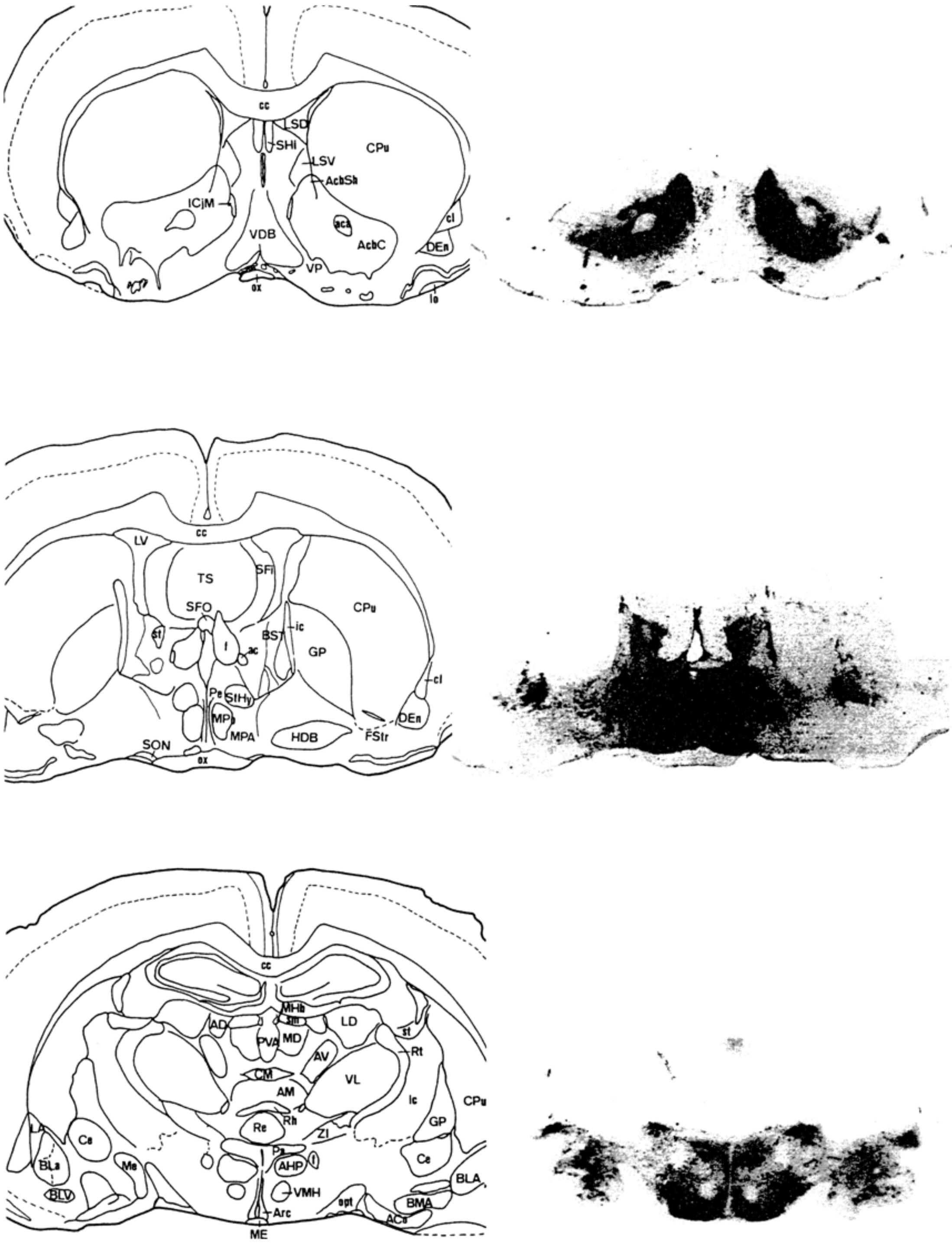
Although binding of CT to brain and other membranes is generally poorly reversible (Fischer et al., 1981a,b; Goltzman, 1980; Findlay et al., 1980), moderate reversibility is observed with sheep brain membranes (Sexton et al., 1991). Molecular sizing of sheep brain CT binding sites crosslinked to a photoactive analog of salmon CT, on SDS-polyacrylamide gel electrophoresis, revealed a single protein of $M^r \sim 72{,}000$ (Fig. 5; Sexton et al., 1991). This is in accord with the molecular weight of CT receptors identified in other tissues (*see* Sexton et al., 1990).

The mechanism of action of central CT receptors is unclear. Unlike CT receptors in kidney, osteoclasts, and various cancer cell lines that utilize cAMP as a second messenger (Nicholson et al., 1987; Findlay et al., 1980; Goltzman, 1980), binding of CT to central CT receptors in rat brain membranes does not stimulate adenylate cyclase activity (Rizzo and Goltzman, 1981; Nicosia et al., 1986; Twery et al., 1988). Indeed salmon CT decreases adenylate cyclase activity at high concentrations (Rizzo and Goltzman, 1981; Nicosia et al., 1986). High concentrations of salmon CT also selectively inhibit the calmodulin-dependent phosphorylation of specific synaptic membrane proteins, suggesting that inhibition of phosphorylation may underlie some of the neural actions of the peptide (Patel et al., 1985). The recent cloning of a CT receptor from renal LLC-PK1 cells (Lin et al., 1991) should form the basis of greater understanding of the molecular mechanism of central CT action in the near future.

Physiological Correlates

Calcitonin is a potent centrally acting analgesic (Guidobono et al., 1986; Morton et al., 1986; Welch et al., 1986; Fabbri et al., 1985; Clementi et al., 1985; Braga et al., 1978). For instance, microinjection of CT into the periaqueductal gray (PAG) or the midline pontine reticular formation increases hot plate latencies (Fabbri et al., 1985), whereas in cats, microinjection into ventral PAG or dorsal raphe magnus decreases C-fiber strength firing of spinal dorsal horn neurons (Morton et al., 1986). These actions correlate well with the location of dense CT binding in ventrolateral PAG and midline raphe nuclei. Further, absence of binding sites in dorsal PAG is in accord with the lack of response to microinjection of CT in this region. The PAG is important in central regulation of pain (Andrezik and Beitz, 1985), and the ventrolateral divisions of the PAG have reciprocal projections to midline raphe nuclei and the pontine and medullary reticular formation, which are enriched in calcitonin binding sites. Thus, CT binding within these regions may also be involved in CT-induced analgesia. The raphe magnus, in particular, is thought to provide a critical relay in the pain suppression path from the ventral PAG (Prieto et al., 1983). Thus, suppression of C-fiber strength firing of dorsal horn neurons following microinjection of CT into the CT receptor-rich raphe magnus provides further evidence for the importance of this nucleus in centrally induced analgesia.

The hypothalamus contains some of the most CT-binding-site-enriched nuclei in the rat brain. This parallels the multiple hypothalamic actions of CT, which include modulation of prolactin (Olgiati et al., 1981; Clementi et al., 1983; Chihara et al., 1982), growth hormone (Tannenbaum and Goltzman, 1985; Lengyel and Tannenbaum , 1987), and adrenocorticotropic hormone release (Rapisarda et al., 1984), as well as decreased appetite (Morimoto et al., 1985; Tannenbaum and Goltzman, 1985; Shimizu and Oomura, 1986; Plata-Salaman and Oomura, 1987: De Beaurepaire and Freed, 1987a), gastric acid secretion (Morley et al., 1981; Sabbatini et al., 1985; Lenz et al., 1986), and intestinal motility (Bueno et al., 1983; Lenz, 1988). The proposed role of the medial basal hypothalamus, arcuate nucleus, and median eminence in the action of calcitonin on prolactin release (Chihara et al., 1982) is supported by the presence of calcitonin binding sites in these structures. ^{125}I-salmon CT injected intracerebroventricularly (icv) binds predominately to the hypothalamus, with minimal binding to neocortex and cerebellum (Morimoto et al., 1985; Sabbatini et al., 1985), where the level of binding is correlated with the inhibition of appetite (Morimoto et al., 1985) and gastric acid secretion (Sabbatini et al., 1985). Development of the anorexic effect was further correlated to structures surrounding the cerebral III ventricle (Shimizu and Oomura, 1986; Plata-Salaman and Oomura, 1987). Mapping studies, using microinjections of peptide, demonstrated most potent inhibition of feeding following injections into the paraventricular nucleus, perifornical area, and parts of the floor of the hypothalamus, with a less marked inhibition with injection into nucleus accumbens and no effect when injected into ventrolateral


V
cc
LSD
SHi
CPu
LSV
AcbSh
ICjM
aca
VDB
AcbC
cl
DEn
VP
ox
lo
cc
LV
TS
SFi
CPu
SFO
BST
ic
st
ac
GP
cl
Pe
StHy
MPo
DEn
MPA
HDB
FStr
SON
ox
cc
MHb
sm
AD
LD
st
PVA
MD
Rt
AV
CM
VL
AM
ic
CPu
Rh
GP
Re
ZI
Ce
Pe
AHP
Ce
Me
BLa
VMH
BLA
BLV
opt
BMA
Arc
ACo
ME

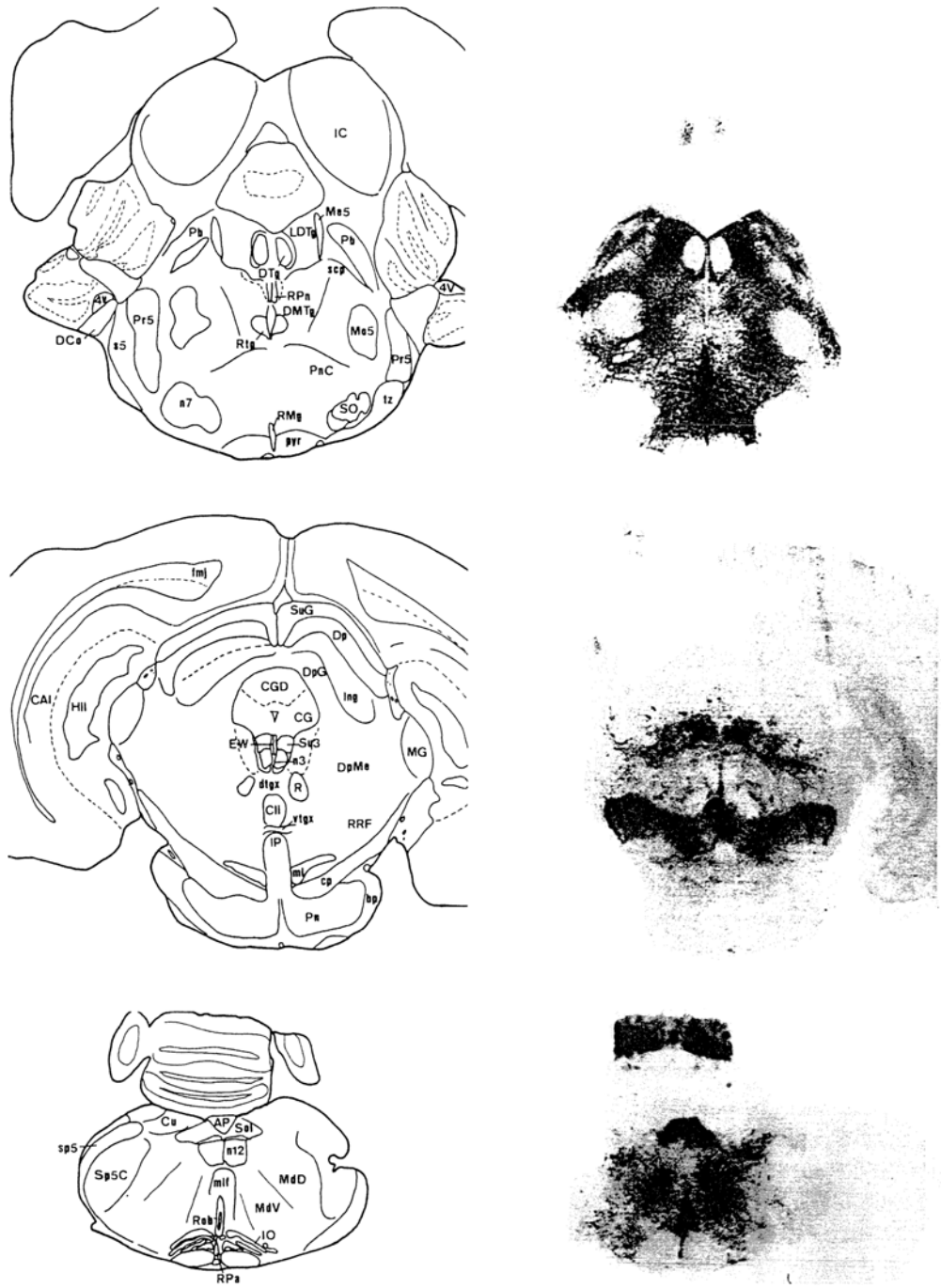


Fig. 4. Autoradiographic mapping of the distribution of ^{125}I-salmon CT binding sites in rat brain. Abbreviations are as described in Attachment 1.

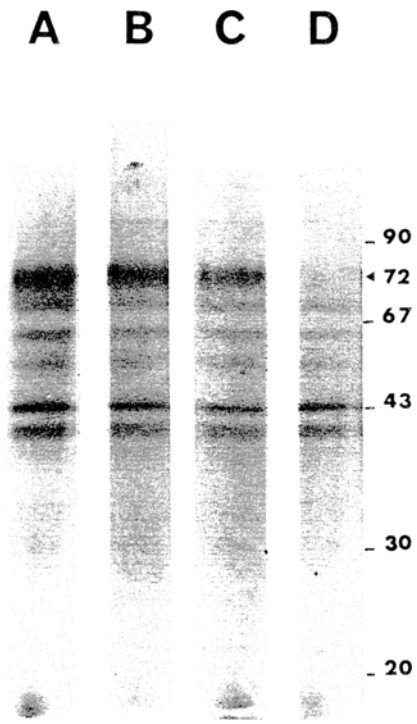


Fig. 5. Photoaffinity crosslinking of CT to binding sites in solubilized sheep brain membranes with ^{125}I-[Arg^{11,18}, 4-azidobenzoyl-Lys^{14}] salmon CT in the presence or absence of increasing concentrations of salmon CT (sCT). Lane A, 0 sCT, Lane B, 0.1 n*M* sCT, Lane C, 1.0 n*M* sCT, Lane D, 300 n*M* sCT.

hypothalamus, posterior hypothalamic nucleus, median forebrain bundle, and olfactory tubule (De Beaurepaire and Freed, 1987a).

Central administration of CT also affects the extrapyramidal motor system, where it antagonizes amphetamine-induced hyperactivity (Twery et al., 1986a; De Beaurepaire and Freed, 1987b), and elicits dyskinesia (Twery et al., 1986b; Morimoto et al., 1985) and catalepsy (Twery et al., 1986b). Since CT increases nigral glutamate decarboxylase (Nicoletti et al., 1982), binding sites present in the substantia nigra pars compacta may represent a target for some of these effects. However, microinjection studies into discrete brain regions show that efficacy of CT in antagonizing amphetamine-induced hyperactivity is greatest in restricted nuclei of the hypothalamus with a lesser effect at the nucleus accumbens (the substantia nigra was not studied), a pattern that paralleled the effects on appetite (De Beaurepaire and Freed, 1987a).

Additional actions of CT include the enhancement of glucose-stimulated release of insulin (Greely et al., 1989), and increases in plasma renin activity and blood pressure (Clementi et al., 1986). Effects on blood pressure may be owing to an interaction with CT sites in the dorsal vagal complex or the parabrachial nuclei, regions involved in central cardiovascular homeostasis.

CT in the Central Nervous System

Although low levels of human CT-like immunoreactivity have been detected in extracts of both rat (Flynn et al., 1981) and human (Fischer et al., 1981b) brain, the virtual lack of mammalian CT mRNA expression in the CNS (Rosenfeld et al., 1983) has invoked questions on the existence of an endogenous ligand for central CT receptors and the physiological significance of these receptors. In nonmammalian species, salmon CT-like immunoreactivity was present in the CNS of lizards, where it was localized by immunocytochemistry to nerve varicosities, and terminals of the hypothalamus and median eminence (MacInnes et al., 1982), and in pigeons (Galan Galan et al., 1981), suggesting that a salmon CT-like molecule may act as a neurotransmitter or neuromodulator in the CNS. Along with human CT, a salmon CT-like peptide has been identified in human brain extracts (Fischer et al., 1983), and as such represents a potential candidate for the endogenous ligand of central CT receptors. The presence of a gene expressing a salmon-like peptide in humans is supported by hybridization of chicken CT cDNA to Northern blots of human medullary carcinoma tissue (Lasmoles et al., 1985). Indeed there is evidence for the existence of multiple types of CT in fish, reptiles, amphibia, mammals and birds (Perez Cano et al., 1981, 1982a,b).

CGRP Binding Sites

Distribution

CGRP binding sites, localized by in vitro autoradiography, have a characteristic distribution that is generally quite distinct from that of CT sites. Moderate to high concentrations of CGRP binding sites in rat brain occur within the ventral nucleus of the diagonal band, nucleus accumbens, OVLT, caudal-ventral putamen, lateral and basolateral amygdaloid nuclei, arcuate nucleus (hypothalamus), lateral mammillary nucleus, habenular nuclei, medial and lateral geniculate nuclei, superior and inferior colliculi, pontine nuclei, most of the cranial nerve nuclei, entorhinal cortex and parts of the visual and auditory cortex, molecular and Purkinje cell layers of the cerebellum, NTS, area postrema, inferior olivary complex, lateral reticular nuclei (medulla), hypoglossal complex, the vestibular and cochlear nuclei, and spinal cord (Fig. 6; Sexton et al., 1986; Seifert et al., 1985; Skofitsch and Jacobowitz, 1985a; Henke et al., 1985). The pattern of binding in human brain is essentially the same (Inagaki et al., 1986).

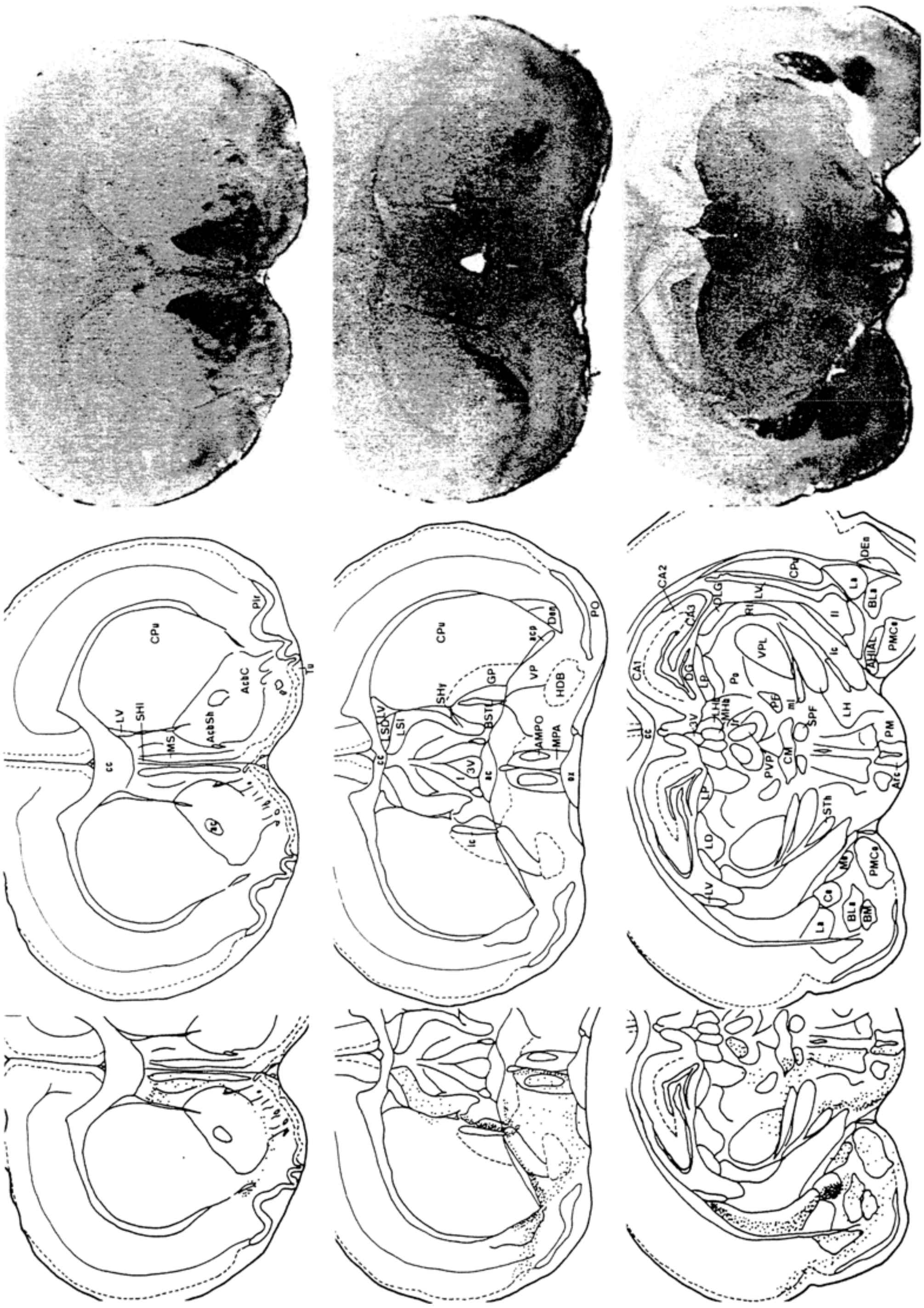
Similar receptor distributions have been reported using both ^{125}I-α- and β-CGRP as radioligands in human brain homogenates, except for the cerebellum and inferior colliculus, which appear to have more α-CGRP binding than β-CGRP binding (Henke et al., 1987). Binding sites for β-CGRP, visualized by in vitro autoradiography, are more diffuse and are present in the ventromedial hypothalamus, which has only low levels of binding when ^{125}I-α-CGRP is used as the radioligand. However, both α- and β-human CGRP have similar efficacy in inhibiting binding of ^{125}I-β-CGRP (Henke et al., 1987). Salmon CT is 500–1000-fold less potent than CGRP in inhibiting binding to sites labeled with either radioligand (Henke et al., 1987).

Properties

In specificity studies using cerebellar cortical membranes, α-rat CGRP, α-human CGRP, and β-human CGRP display high-affinity binding, whereas Cys(ACM)^{2,7} CGRP and the C-terminal (23–37) fragment have little potency (Sexton et al., 1986; Seifert et al., 1985). Salmon CT and its des Ser^2 analog do not compete except at high concentrations (up to 1000-fold greater) (Sexton et al., 1986). Unlike CT binding, binding of CGRP to its binding site is rapidly reversible (Seifert et al. 1985; Yoshizaki et al., 1987). Crosslinking studies in human cerebellum with either α- or β-^{125}I human CGRP revealed specific proteins of M_r ~ 17,500 and 54,000 (Dotti-Sigrist et al., 1988). In other tissues, CGRP specific binding proteins of M_r 60,000 (in rat smooth muscle cells and bovine endothelial cells; Hirata et al., 1988) and M_r 120,000, 90,000, and 70,000 (in pig coronary arteries, heart atrium, and ventricular muscles; Miyauchi et al., 1988; Sano et al., 1989) have been recognized. Gel filtration studies of CGRP binding sites solubilized from porcine spinal cord revealed an M_r of ~ 400,000 (Hiroshima et al., 1988), indicating either the presence of oligomeric forms of the protein or close association of additional proteins.

Recently Dennis et al. (1989,1990) have indicated the existence of CGRP receptor subtypes. They propose two CGRP receptor subtypes denoted CGRP-I and CGRP-II, where type-I receptors are more potently antagonized by the antagonist peptide human CGRP$_{8-37}$, with CGRP-II receptors being poorly antagonized by CGRP$_{8-37}$ and more potently stimulated by the analogs Cys(ACM)$_{2,7}$ human CGRP and Tyr^0 human CGRP. In support of this, the central action of CGRP to inhibit food intake is potently antagonized by the 8–37 peptide, whereas CGRP-induced hyperthermia is not antagonized (Dennis et al., 1990). However, CGRP$_{8-37}$ was equipotent in inhibiting ^{125}I-human CGRP binding, in both atrial (type-I "receptor") and vas deferens (type-II "receptor") membranes, rendering the underlying mechanism of the pharmacological observations obscure.

Examination of changes in CGRP binding with age revealed selective decreases in binding density (Guidobono et al., 1989). Thus, marked reduc-



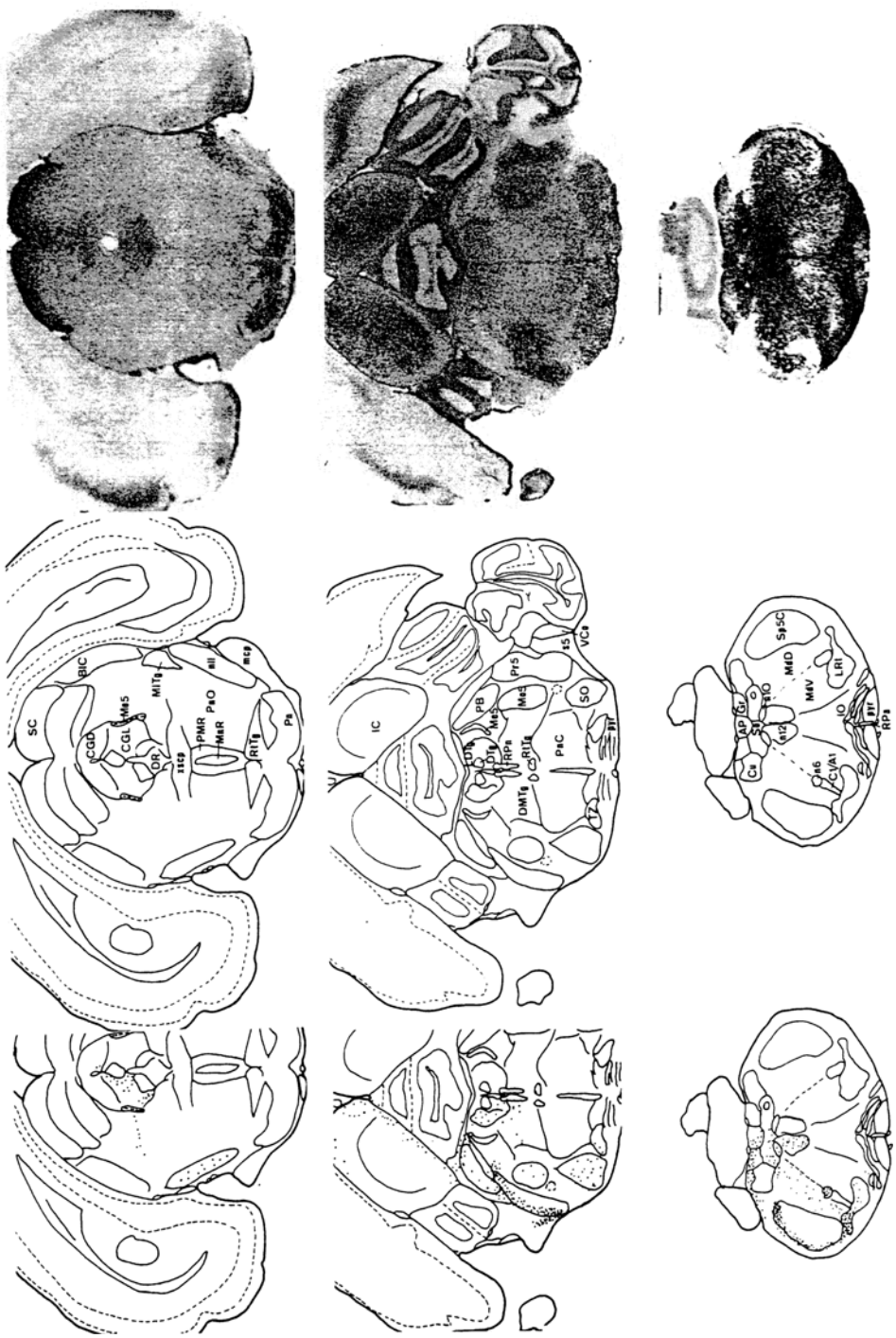


Fig. 6. Autoradiographic mapping of the distribution of ^{125}I-rat CGRP α binding sites in rat brain (right-hand panel, adapted from Sexton et al., 1986). The left-hand panel illustrates patterns of CGRP-immunoreactive fiber distribution (adapted from Kawai et al., 1985; Skofitsch and Jacobowitz (1985).

tions occurred in the hippocampus, the nucleus rhomboideus, arcuate nucleus, superior colliculus, central substantia grisea, and the spinal cord, whereas binding to the cortical areas, the amygdala, caudate putamen, and nucleus accumbens was not modified. Binding in the cerebellum was increased. Although the molecular basis for changes in binding density is unknown, it may be related to selective natural loss of brain function, changes in CGRP-fiber innervation, or perhaps the biochemical properties of the receptors themselves (*see below*).

Physiological Correlates

Intracerebroventricular injection of CGRP raises blood pressure and heart rate, which, at high concentrations, is paralled by an increase in sympathetic noradrenergic outflow (Fisher et al., 1983), supporting a role for CGRP in central cardiovascular regulation. This is consistent with the localization of CGRP peptide and binding sites in the NTS, parabrachial nuclei, and hypothalamus. Further, direct injection of rat CGRP into the NTS produced dose-dependent changes in blood pressure, whereas low and subthreshold doses modified the cardiovascular response to NTS injection of noradrenaline (Vallejo et al., 1988), suggesting that at least some of the CGRP binding sites in the NTS are involved in cardiovascular homeostasis. CGRP binding in the NTS, however, is unaltered by nodose ganglionectomy (in contrast to angiotensin binding sites), demonstrating that the receptors are not presynaptic on primary vagal afferents (Allen et al., 1987).

In the hypothalamus, CGRP receptors may be involved in decreasing appetite and growth hormone secretion (Krahn et al. 1984; Tannenbaum and Goltzman, 1985), and modulating gastrointestinal function where icv CGRP inhibits gastric acid (Lenz et al., 1985; Tache et al., 1984) and bicarbonate (Lenz and Brown, 1990) secretion and intestinal motility (Fargeas et al., 1985; Lenz, 1988). However, the association of CGRP binding sites with gustatory afferent pathways (*see below*) also suggests that CGRP may have indirect effects on appetite through taste.

CGRP binding is associated with most of the cranial nerve nuclei, and is colocalized with choline acetyl transferase in neurons of the 6th, 7th, and 12th nuclei, as well as in neuromuscular junctions of striated muscle (Rodrigo et al., 1985; Takami et al., 1985a) where it enhances neuromuscular contraction (Takami et al., 1985b). CGRP also acts via cAMP to increase acetylcholine receptor (AChR) number and AChR α-subunit mRNA in chick myotubes (Fontaine et al., 1989). These findings suggest that CGRP may interact with cholinergic neurons to influence motor control.

In addition, the pattern of CGRP binding reveals association with several functional systems. This association is striking in the auditory system, with binding sites localized in the cochlear nuclei, superior olivary nuclei, nuclei of the lateral lemniscus, central nucleus of the inferior colliculus, medial geniculate nucleus, and the middle or receptive layers of the auditory cortex. Also in the gustatory system (Braun et al., 1982), binding sites are found in the NTS, the parabrachial nuclei, posterior ventromedial thalamic nuclei, central amygdaloid nucleus, and in the gustatory cortex. In the olfactory system, there is low binding over the mitral cell dendrites of the olfactory bulb, a moderate density in primary and secondary (entorhinal) olfactory cortex, and moderate to high binding densities in parts of the amygdala. CGRP, in the olfactory bulb, is involved in the differentiation of dopaminergic neurons (Denis-Donini, 1989) and may have additional trophic effects in other systems. Nonetheless, these patterns of binding suggest that CGRP may be involved in modulating a number of special sensory pathways.

Correlation with CGRP Immunoreactivity

CGRP-immunoreactive cells and fibers are discretely distributed throughout the CNS (Kawai et al., 1985; Skofitsch and Jacobowitz, 1985b, Kruger et al., 1988). Comparison of the distribution of CGRP-binding sites and the reported location of CGRP-immunoreactive fibers demonstrates

a definite correlation (Fig. 6). This is evident in some of the sensory and somatosensory pathways with CGRP-immunoreactive fibers and receptors in the sensory nuclei of the trigeminal nerve, the cuneate, and gracile nuclei, and the NTS. There are, however, some discrepancies in the relative densities of innervation and binding, as illustrated by the amygdala where fiber densities are highest in the lateral portion of the central amygdala, with only moderate to low fiber density in the basolateral and lateral divisions, which contain high receptor densities. Kruger et al. (1988) suggest that this may parallel the CGRP innervation of peripheral tissues where direct synapses are lacking, and that neither the locus of immunoreactive-axon terminals nor binding sites need indicate transmitter action for impulse information transfer. Major differences include adult cerebellum, the pontine nuclei, and the inferior olive where high binding densities occur, whereas there is an absence of CGRP-immunoreactive fibers (Fig. 6; Sexton et al., 1986; Kawai et al., 1985; Skofitsch and Jacobowitz, 1985a,b; Tschopp et al., 1985).

CT-Sensitive CGRP Binding Sites

Properties and Distribution

Mapping of central CT and CGRP binding sites, and comparison of relative distribution reveal partial overlap in the patterns of binding in parts of the forebrain (Sexton et al., 1988), although in most brain regions, binding of the two peptides is biochemically and morphologically distinct. However, in some restricted regions, such as the nucleus accumbens, there is high-affinity binding of both CT and CGRP. In this nucleus, salmon CT competed for CGRP-labeled sites with similar affinity to unlabeled CGRP (Fig. 7A), suggesting the existence of a new subclass of binding site (termed C3; Sexton et al., 1988) that has high affinity for both peptides (Sexton et al., 1988; Dennis et al., 1991). This binding site specificity is in marked contrast to binding at conventional CT (*see* CT Binding Sites) or CGRP binding sites, including cerebellum (*see* CGRP Binding Sites) and primary olfactory cortex (Fig. 7B). Studies with $CGRP_{8-37}$ and $Cys(ACM)^{2,7}$ CGRP (Dennis et al., 1991) indicate that the "C3" site has distinct specificity from the CGRP-I and CGRP-II receptor subtypes proposed by Dennis et al. (1989,1990). The "C3" sites, localized by digital subtraction autoradiography, have a restricted distribution in the CNS, including parts of the ventral striatum, lateral bed nucleus of the stria terminalis, central amygdaloid nucleus, dorsal raphe nucleus, OVLT, and area postrema (Fig. 8; Sexton et al., 1988). Studies of binding in the nucleus accumbens across species suggest that the presence of "C3" binding sites is a species-specific phenomenon (Dennis et al., 1991). Ontogenic studies in rats indicate that binding to the nucleus accumbens increases progressively after postnatal day 4 and is only 50% of adult levels (>3 mo) after 1 mo, whereas CGRP binding sites in the neighboring primary olfactory cortex have reached adult levels by postnatal day 7 (Dennis et al., 1991). The levels of binding in the adult nucleus accumbens (and also central amygdaloid nucleus) remain constant with aging (22 mo old), whereas binding to some conventional CGRP sites (e.g., arcuate nucleus, superior colliculus) is markedly decreased (Guidobono et al., 1989). It must be noted, however, that many other CGRP binding sites are undiminished with age (*see* CGRP Binding Sites) (Guidobono et al., 1989), indicating that changes in binding-site density with age are more likely to be influenced by other factors (e.g., changes in density of CGRP-immunoreactive fibers) rather than by the biochemical properties of the binding site.

Given the high degree of discrimination in binding between CT and CGRP at conventional receptors, the interaction of CT and CGRP at "C3" binding sites represents a novel interaction for such biochemically diverse peptides. There are many cases of structurally related peptides sharing common receptors with similar potency, e.g., the two forms of interleukin 1 (IL1) at IL1 receptors (Bird and Saklatvala, 1986; Dower et al., 1986), and parathyroid hormone (PTH) and PTH-related protein at PTH receptors (Kemp et al., 1987).

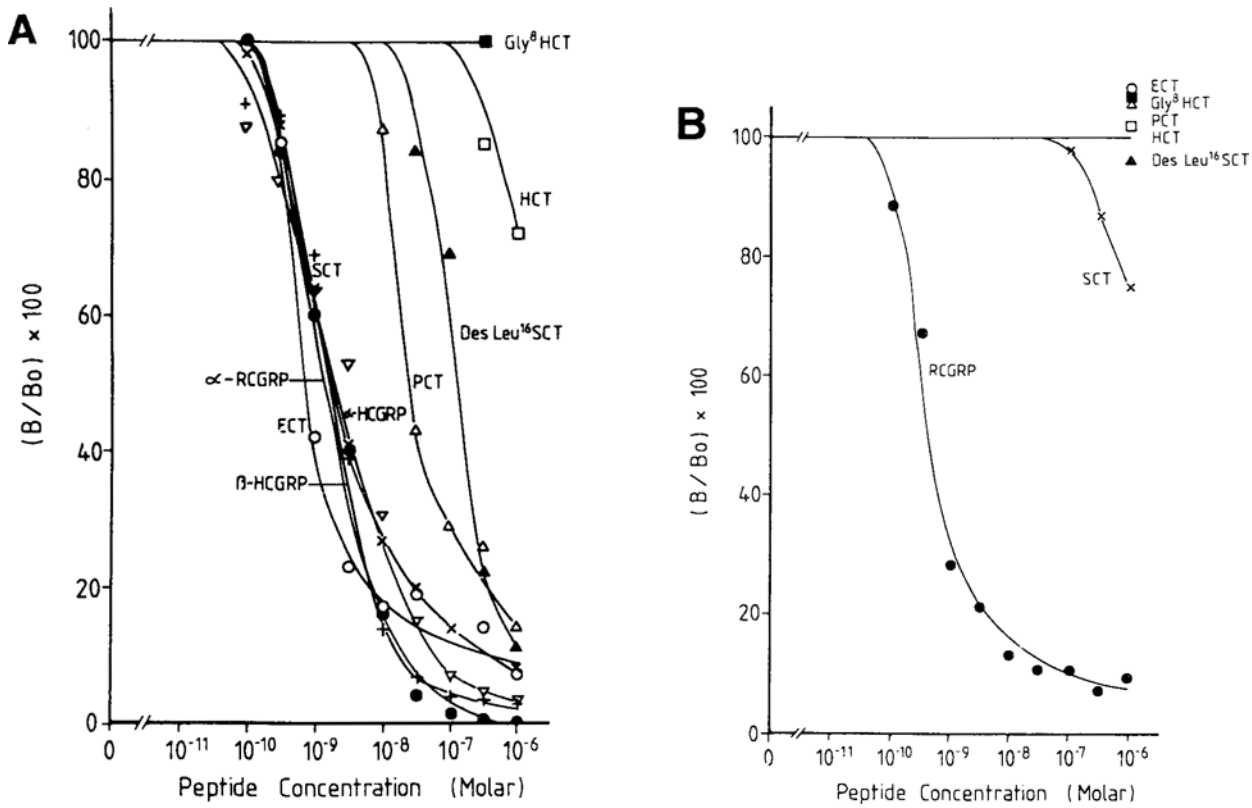


Fig. 7. **A.** Competition of ^{125}I-rat CGRP α binding to sections of nucleus accumbens by CT and CGRP peptides. **B.** Competition of ^{125}I-rat CGRP binding to sections of primary olfactory cortex by CT and CGRP peptides (from Sexton et al., 1988).

However, receptor discrimination is common even among structurally related peptides, e.g., insulin, insulin-like growth factor (IGF) 1, and IGF2, which have separate receptors and a complex pattern of interaction (Czech, 1982). Furthermore, discrete receptor specificity may be maintained despite up to 90% homology between ligands, e.g., human growth hormone and human placental lactogen at their respective receptors (Watahiki et al., 1989; Hill et al., 1988).

Parallels between the interaction of CT and CGRP at "C3" binding sites may be drawn with transforming growth factor α (TGF α) and epidermal growth factor (EGF), where both bind to the EGF receptor with similar efficacy (Tam et al., 1984). Despite low direct amino acid homology, both TGF α and EGF have conserved tertiary structure through six cysteine residues similarly placed within the peptides and 60% homology in the loop region between the fifth and sixth cysteines, which is believed to be involved in binding (Derynck, 1988). A similar case could be made for the interaction of salmon CT and rat CGRP α at "C3" binding sites. Although there is <30% homology between the two peptides (Fig. 2 vs 3), both have an amino terminal disulfide bridged loop structure that shares 50% homology and a region of α-helical propensity (Breeze et al., 1991; Meadows et al., 1991). The amino-terminal region of CT is thought to be involved in binding of the hormone to its receptor (*see* Orlowski et al., 1987), and this may explain similarities in the CT binding specificities of conventional CT binding sites and "C3" binding sites (Sexton et al., 1988).

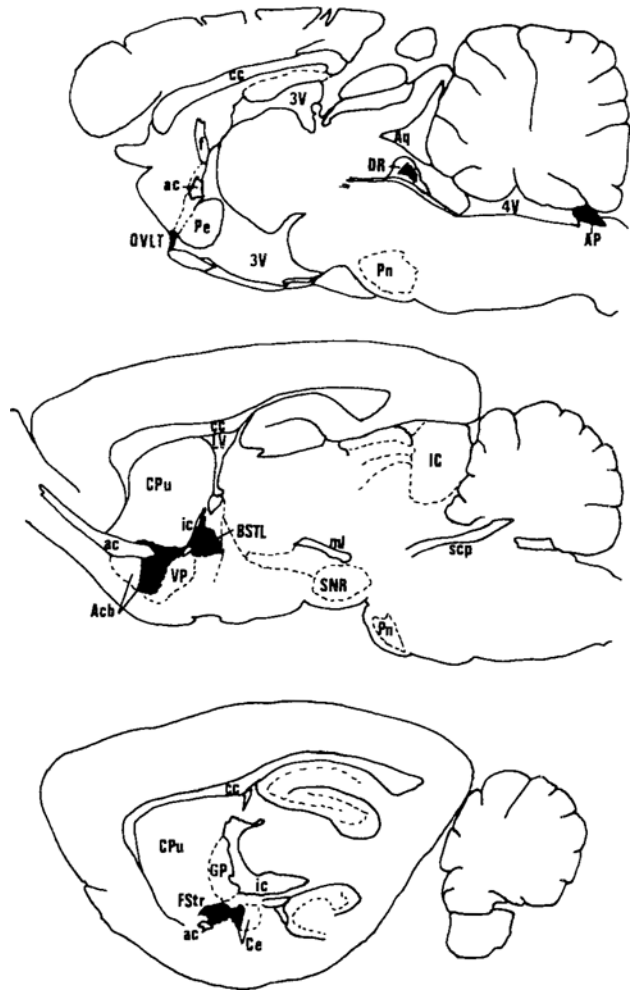


Fig. 8. Schematic diagram of the distribution of "C3" binding sites.

Nonetheless, unlike TGF α and EGF, CT and CGRP demonstrate discrete biochemical specificity at conventional CT and CGRP receptors.

The significance of this new class of binding site, which recognizes both peptides with equal potency, is not clear. The regional distribution of "C3 " sites is much more restricted than either CT or CGRP binding sites. The structures containing the "C3" binding sites are discretely localized and do not correspond to a single functional system. However, the pattern of distribution of binding sites through the nucleus accumbens, lateral bed nucleus of the stria terminalis, fundus striati, and central nucleus of the amygdala (Sexton et al., 1988), is similar to the pattern of distribution of ascending CGRP-immunoreactive fibers in these nuclei (Kawai et al., 1985; Skofitsch and Jacobowitz, 1985b). This suggests that "C3 " sites in these structures will interact with neuronal CGRP and may be involved with specific brainstem projections.

Also, "C3" binding sites were found in two of the circumventricular organs, the OVLT and the area postrema. Not all the circumventricular organs are labeled, however, since the subfornical

organ is enriched with only CT binding sites. The circumventricular organs bind circulating CT (Van Houten et al., 1982), and CGRP-immunoreactive fibers terminate in both the OVLT and area postrema (Kawai et al., 1985; Skofitsch and Jacobowitz, 1985b). It is therefore possible that "C3" binding sites in these regions provide an area of interaction between neuronal CGRP and circulating CT. In the hypothalamus, we detected abundant CT binding sites and a moderate density of CGRP binding sites, but no "C3" sites (Sexton et al., 1988), indicating that "C3" binding sites are not an invariable consequence of overlap in CT and CGRP binding distribution.

It is possible that "C3" binding sites represent an evolutionary intermediate in the development of CT receptors from CGRP receptors (or vice versa), with the new receptor arising from modification of a redundant copy of the original receptor. Similar mechanisms have been proposed for the evolution of peptide hormones (Niall, 1982). The retention of a binding domain that binds both hormones suggests that, in the brain regions where it is found, there is little selective pressure to discriminate between CT and CGRP. Conversely, in other regions, both centrally and peripherally, there must have been considerable selective pressure to develop discrete signals to influence, for instance, calcium regulation (CT) and vascular tone (CGRP).

Conclusion

Binding sites for CT and CGRP are widely distributed in the central nervous system in characteristic patterns. They subserve a diverse range of actions consistent with their localization, although the actual physiological roles of the binding sites are unclear. However, the advent of antagonist peptides for CGRP receptors and molecular cloning of the CT receptor should enable a greater understanding of the physiological roles of the receptors, the relationships between CT and CGRP receptors (and receptor subtypes), and their mechanisms of action, in the near future.

Acknowledgments

This work was supported in part by grants from the National Health and Medical Research Council of Australia and the Australian Research Council. P. M. S. is an Australian Research Council Post Doctoral Research Fellow. The author thanks Joanne Hilton for her aid in preparation of this manuscript.

Attachment 1

Neuroanatomical Abbreviations Used in Figures

3V	Third ventricle
4V	Fourth ventricle
ac	Anterior commissure
AcbC	Accumbens nucleus, core
AcbSh	Accumbens nucleus, shell
ACo	Anterior cortical amygdaloid nucleus
acp	Anterior commissure, posterior part
AD	Anterodorsal thalamic nucleus
AHiAL	Amygdalohippocampal area, anterolateral part
AHP	Anterior hypothalamic area, posterior part
AM	Anteromedial thalamic nucleus
AMPO	Anterior medial preoptic nucleus
AP	Area postrema
Arc	Arcuate hypothalamic nucleus
AV	Anteroventral thalamic nucleus
BIC	Nucleus of the brachium of the inferior colliculus
BLa	Basolateral amygdaloid nucleus, anterior part
BLV	Basolateral amygdaloid nucleus, ventral part
BM	Basomedial amygdaloid nucleus
BMA	Basomedial amygdaloid nucleus, anterior part
bp	Brachium pontis (stem of middle cerebellar peduncle)
BST	Bed nucleus of the stria terminalis

BSTL	Bed nucleus of the stria terminalis, lateral division
CA1–4	Fields CA1–4 of Ammon's horn
cc	Corpus callosum
Ce	Central amygdaloid nucleus
CG	Central (periaqueductal) gray
CGD	Central gray (dorsal part)
CGL	Central gray (lateral part)
cl	Claustrum
CLi	Caudal linear nucleus of the raphe
CM	Central medial thalamic nucleus
CM	Central medial thalamic nucleus
cp	Cerebral peduncle, basal part
CPu	Caudate putamen
Cu	Cuneate nucleus
DEn	Dorsal endopiriform nucleus
DG	Dentate gyrus
DLG	Dorsal lateral geniculate nucleus
DMTg	Dorsomedial tegmental area
DpG	Deep gray layer of the superior colliculus
DpMe	Deep mesencephalic nucleus
DR	Dorsal raphe nucleus
DTg	Dorsal tegmental nucleus
dtgx	Dorsal tegmental decussation
EW	Edinger-Westphal nucleus
f	Fornix
fi	Fimbria of the hippocampus
fmj	Forceps major of the corpus callosum
fr	Fasciculus retroflexus
FStr	Fundus striati
GP	Globus pallidus
Gr	Gracile nucleus
HDB	Nucleus of the horizontal limb of the diagonal band
Hil	Hilus of the dentate gyrus
ic	Internal capsule
IC	Inferior colliculus
ICjM	Islands of Calleja, major island
Ing	Intermediate gray layer of the superior colliculus
IO	Inferior olive
IP	Interpeduncular nucleus
La	Lateral amygdaloid nucleus
LA	Lateral amygdaloid nucleus
LD	Laterodorsal thalamic nucleus
LDTg	Laterodorsal tegmental nucleus
LH	Lateral hypothalamic area
LHb	Lateral habenular nucleus
lo	Lateral olfactory tract
LP	Lateral posterior thalamic nucleus
LRt	Lateral reticular nucleus
LSD	Lateral septal nucleus, dorsal part
LSI	Lateral septal nucleus, intermediate part
LSV	Lateral septal nucleus, ventral part
LV	Lateral ventricle
mcp	Middle cerebellar peducle
MD	Mediodorsal thalamic nucleus
MdD	Medullary reticular nucleus, dorsal part
MdV	Medullary reticular nucleus, ventral part
Me	Medial amygdaloid nucleus
Me5	Mesencephalic trigeminal nucleus
MG	Medial geniculate nucleus
MHb	Medial habenular nucleus
MiTg	Microcellular tegmental nucleus
ml	Medial lemniscus
mlf	Medial longitudinal fasciculus
MnR	Median raphe nucleus
Mo5	Motor trigeminal nucleus
MPA	Medial preoptic area
MPo	Medial preoptic nucleus
MS	Medial septal nucleus
n3	Oculomotor nucleus
n6	Abducens nucleus
n7	Facial nucleus
n10	Dorsal motor nucleus, vagus
n11	Nucleus of the lateral lemniscus
n12	Hypoglossal nucleus
opt	Optic tract
ox	Optic chiasm
Pa	Paraventricular hypothalamic nucleus
Pb	Parabrachial nuclei
Pe	Periventricular hypothalamic nucleus
PF	Parafascicular thalamic nucleus
PM	Premammillary nucleus
PMCo	Posteromedial cortical amygdaloid nucleus (C3)
PMR	Paramedian raphe nucleus

Pnl	Pontine nucleus
PnC	Pontine reticular nucleus, caudal part
PnO	Pontine reticular nucleus, oral part
Po	Primary olfactory cortex
Pr5	Principal sensory trigeminal nucleus
PVA	Paraventricular thalamic nucleus, anterior part
PVP	Paraventricular thalamic nucleus, posterior part
pyr	Pyramidal tract
R	Red nucleus
Re	Reuniens thalamic nucleus
Rh	Rhomboid thalamic nucleus
RMg	Raphe magnus nucleus
Rob	Raphe obscurus nucleus
RPa	Raphe pallidus nucleus
RPn	Raphe pontis nucleus
Rr	Retrorubral nucleus
RRf	Retrorubral field
Rt	Reticular thalamic nucleus
RtTg	Reticulotegmental nucleus, pons
s5	Sensory root of the trigeminal nerve
SC	Superior colliculus
scp	Superior cerebellar peduncle
SFi	Septofimbrial nucleus
SFO	Subfornical organ
SHi	Septohippocampal nucleus
SHy	Septohypothalamic nucleus
sm	Stria medullaris of the thalamus
SO	Superior olivary nucleus
Sol	Nucleus of the solitary tract
SON	Supraoptic nucleus
Sp5	Spinal trigeminal tract
Sp5C	Spinal trigeminal nucleus, caudal part
SPF	Subparafascicular thalamic nucleus
st	Stria terminalis
STh	Subthalamic nucleus
StHy	Striohypothalamic nucleus
Su3	Supraoculomotor central gray
SuG	Superficial gray layer, superior colliculus
TS	Triangular septal nucleus
Tu	Olfactory tubercule
Tz	Nucleus of the trapezoid body
tz	Trapezoid body
VCo	Ventral cochlear nucleus
VDB	Nucleus of the vertical limb of the diagonal band
VL	Ventrolateral thalamic nucleus
VMH	Ventromedial hypothalamic nucleus
VP	Ventral pallidum
VPL	Ventral posterolateral thalamic nucleus
vtgx	Ventral tegmental decussation
xscp	Decussation of the superior cerebellar peduncle
ZI	Zona incerta

References

Allen A. M., Chai S.-Y., Sexton P. M., Lewis S. J., Verberne A. J. M., Jarrott B., Louis W. J., Clevers J., McKinley M. J., Paxinos G., and Mendelsohn F. A. O. (1987) Angiotensin II receptors and angiotensin converting enzyme in the medulla oblongata. *Hypertension* **(Suppl. III) 9,** 198–205.

Amara S. G., Evans R. M., and Rosenfeld M. G. (1984) Calcitonin/calcitonin gene-related peptide transcription unit: tissue-specific expression involves selective use of alternative polyadenylation sites. *Mol. Cell Biol.* **4,** 2151–2160.

Amara S. G., Jonas V., Rosenfeld M. G., Ong E. S., and Evans R. M. (1982) Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature* **298,** 240–244.

Amara S. G., Arriza J. L., Leff S. E., Swanson L. W., Evans R. M., and Rosenfeld M. G. (1985) Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide. *Science* **229,** 1094–1097.

Andrezik J. A. and Beitz A. J. (1985) Reticular formation, central gray and related tegmental nuclei. *The Rat Central Nervous System, vol. 2. Hindbrain and Spinal Cord.* Paxinos G., ed., Academic, Australia, pp. 1–28.

Bird T. A. and Saklatvala J. (1986) Identification of a common class of high affinity receptors for both types of porcine interleukin-1 on connective tissue cells. *Nature* **324,** 263–266.

Braga P., Ferri S., Santagostino A., Olgiati V. R., and Pecile A. (1978) Lack of opiate receptor involvement in centrally induced calcitonin analgesia. *Life Sci.* **22,** 971–978.

Braun J. J., Lasiter P. S., and Keifer S. W. (1982) The gustatory neocortex of the rat. *Physiol. Psychol.* **10**, 13–45.

Breeze A. L., Harvey T. S., Bazzo R., and Campbell I. D. (1991) Solution structure of human calcitonin gene-related peptide by proton NMR and distance geometry with restrained molecular dynamics. *Biochemistry* **30**, 575–582.

Bueno L., Fioramont J., and Ferre J. P. (1983) Calcitonin—C.N.S. action to control the pattern of intestinal motility. *Peptides* **4**, 63–66.

Chihara K., Iwasaki J., Iwasaki Y., Minamitani N., Kaji H., and Fujita T. (1982) Central nervous system effect of calcitonin: stimulation of prolactin release in rats. *Brain Res.* **248**, 331–339.

Clementi G., Nicoletti F., Patacchioli F., Prato A., Patti F., Fiore C. E., Matera M., and Scapagnini V. (1983) Hypoprolactinemic action of calcitonin and the tuberoinfundibular dopaminergic system. *Neurochemistry* **40**, 885,886.

Clementi G., Amico-Roxas M., Rapisarda E., Caruso A., Prato A., Trombadore S., Priolo G., and Scapagnini U. (1985) The analgesic activity of calcitonin and the central serotonergic system. *Eur. J. Pharmacol.* **108**, 71–75.

Clementi G., Rapisarda E., Fiore C. E., Prato A., Amico-Roxas M., Millia C., Bernardini R., Maugeri S., and Scapagnini U. (1986) Effects of salmon calcitonin on plasma renin activity and systolic blood pressure in the rat. *Neurosci. Lett.* **66**, 351–355.

Czech M. P. (1982) Structural and functional homologies in the receptors for insulin and insulin-like growth factors. *Cell* **31**, 8–10.

De Beaurepaire R. and Freed W. J. (1987a) Anatomical mapping of the rat hypothalamus for calcitonin induced anorexia. *Pharmacol. Biochem. Behav.* **27**, 177–182.

De Beaurepaire R. and Freed W. J. (1987b) Regional localization of the antagonism of amphetamine-induced hyperactivity by intracerebral calcitonin injections. *Pharmacol. Biochem. Behav.* **27**, 183–186.

Denis-Donini S. (1989) Expression of dopaminergic phenotypes in the mouse olfactory bulb induced by the calcitonin gene-related peptide. *Nature* **339**, 701–703.

Dennis T., Fournier A., St-Pierre S., and Quirion R. (1989) Structure–activity profile of calcitonin gene-related peptide in peripheral and brain tissues. Evidence for receptor multiplicity. *J. Pharmacol. Exp. Ther.* **251**, 718–725.

Dennis T., Fournier A., Guard S., St-Pierre S., and Quirion R. (1991) Calcitonin gene-related peptide (hCGRP-alpha) binding sites in nucleus accumbens. Atypical structural requirements and marked phylogentic differences. *Brain Res.* **539**, 59–66.

Dennis T., Fournier A., Cadieux A., Pomerleau F., Jolicoeur F. B., St-Pierre S., and Quirion R. (1990) $HCGRP_{8-37}$ a calcitonin gene-related peptide antagonist revealing CGRP receptor heterogeneity in brain and periphery. *Pharmacol. Exp. Ther.* **254**, 123–128.

Derynck R. (1988) Transforming growth factor x. *Cell* **54**, 593–595.

Dotti-Sigrist S., Born W., and Fischer J. A. (1988) Identification of a receptor for calcitonin gene-related peptides I and II in human cerebellum. *Biochem. Biophys. Res. Commun.* **151**, 1081–1087.

Dower S. K., Kronheim S. R., Hopp T. P., Cantrell M., Deeley M., Gillis S., Henney C. S., and Urdal D. L. (1986) The cell surface receptors for interleukin-1 alpha and interleukin-1 beta are identical. *Nature* **324**, 266–268.

Elalouf J. M., Roinel N., and de Rouffignac C. (1986) Effects of human calcitonin on water and electrolyte movements in rat juxtamedullary nephrons: inhibition of medullary K recycling. *Pflugers Arch.* **406**, 502–508.

Fabbri A., Fraioli F., Pert C. B., and Pert A. (1985) Calcitonin receptors in the rat mesencephalon mediate its analgesic actions: autoradiographic and behavioural analyses. *Brain Res.* **343**, 205–215.

Fargeas M. J., Fioramonti J., and Bueno L. (1985) Calcitonin gene-related peptide: brain and spinal action on intestinal motility. *Peptides* **6**, 1167–1171.

Findlay D. M., de Luise M., Michelangeli V. P., Ellison M., and Martin T. J. (1980) Properties of a calcitonin receptor and adenylate cyclase in a human cancer cell line (BEN cells). *Cancer Res.* **40**, 1311–1317.

Fischer J. A., Sagar S. M., and Martin J. B. (1981a) Characterization and regional distribution of calcitonin binding sites in the rat brain. *Life Sci.* **29**, 663–671.

Fischer J. A., Tobler P. H., Kaufmann M., Born W., Henke H., Cooper P. E., Sagar S. M., and Martin J. B. (1981b) Calcitonin: regional distribution of the hormone and its binding sites in the human brain and pituitary. *Proc. Natl. Acad. Sci. USA* **78**, 7801–7805.

Fischer J. A., Tobler P. H., Henke H., and Tschopp F. A. (1983) Salmon and human calcitonin-like peptides coexist in the human thyroid and brain. *Clin. Endocrinol. Metab.* **57**, 1314–1316.

Fisher L. A., Kikkawa D. O., Rivier J. E., Amara S. G., Evans R. M., Rosenfeld M. G., Vale W. W., and Brown M. R. (1983) Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide. *Nature* **305,** 534–536.

Flynn J. J., Margules D. L., and Cooper C. W. (1981) Presence of immunoreactive calcitonin in the hypothalamus and pituitary lobes of rats. *Brain Res. Bull.* **6,** 547–549.

Fontaine B., Klarsfeld A., Laufer R., Hokfelt T., and Changeux J. P. (1989) Role trophique possible sur la jonction neuromusculaire d'un neuropeptide coexistant avec l'acetylcholine dans les neurones moteurs de la moelle epiniere. *Rev. Neurol. (Paris)* **145,** 194–200.

Galan Galan F., Rogers R. M., Girgis S. I., and MacIntyre I. (1981) Immunoreactive calcitonin in the central nervous system of the pigeon. *Brain Res.* **212,** 59–66.

Goltzman D. (1980) Examination of interspecies differences in renal and skeletal receptor binding and adenylate cyclase stimulation with human calcitonin. *Endocrinology* **106,** 510–518.

Goltzman D. and Mitchell J. (1985) Interaction of calcitonin and calcitonin gene-related peptide at receptor sites in target tissues. *Science* **227,** 1343–1345.

Greely G. H., Cooper C. W., Jeng Y.-J., Eldridge J. C., and Thompson J. C. (1989) Intracerebroventricular administration of calcitonin enhances glucose-stimulated release of insulin. *Regul. Peptides* **24,** 259–268.

Guidobono F., Netti C., and Pecile A. (1987) Calcitonin binding site distribution in the cat central nervous system: a wider insight of the peptide involvement in brain functions. *Neuropeptides* **10,** 265–273.

Guidobono F., Netti C., Sibilia V., Zamboni A., and Pecile A. (1986) Eel calcitonin binding site distribution and antinociceptive activity in rats. *Peptides* **7,** 315–322.

Guidobono F., Netti C., Bettica P., Sibilia V., Pagani F., Cazzamalli E., and Pecile A. (1989) Effects of age on binding sites for calcitonin gene-related peptide in the rat central nervous system. *Neurosci. Lett.* **102,** 20–26.

Henke H., Tobler P. H., and Fischer J. A. (1983) Localization of salmon calcitonin binding sites in rat brain by autoradiography. *Brain Res.* **272,** 373–377.

Henke H., Tschopp F. A., and Fischer J. A. (1985) Distinct binding sites for calcitonin gene-related peptide and salmon calcitonin in rat central nervous system. *Brain Res.* **360,** 165–171.

Henke H., Sigrist S., Lang W., Schneider J., and Fischer J. A. (1987) Comparison of binding sites for the calcitonin gene-related peptides I and II in man. *Brain Res.* **410,** 404–408.

Hill D. J., Freemark M., Strain A. J., Handwerger S., and Milner R. D. G. (1988) Placental lactogen and growth hormone receptors in human fetal tissues: relationships to fetal plasma human placental lactogen concentrations and fetal growth. *J. Clin. Endocrinol. Metab.* **66,** 1283–1290.

Hirata Y., Takagi Y., Takata S., Fukuda Y., Yoshimi H., and Fujita T. (1988) Calcitonin gene-related peptide receptor in cultured vascular smooth muscle and endothelial cells. *Biochem. Biophys. Res. Commun.* **151,** 1113–1121.

Hiroshima O., Sano Y., Yuzuriha T., Yamato C., Saito A., Okamura N., Uchiyama Y., Kimura S., and Goto K. (1988) Solubilization and characterization of calcitonin gene-related peptide binding site from porcine spinal cord. *J. Neurochem.* **50,** 480–485.

Inagaki S., Kito S., Kubota Y., Girgis S. I., Hillyard C. J., and MacIntyre I. (1986) Autoradiographic localization of calcitonin gene-related peptide binding sites in human and rat brains. *Brain Res.* **374,** 287–298.

Kawai Y., Takami K., Shiosaka S., Emson P. C., Hillyard C. J., Girgis S. I., MacIntyre I., and Tohyama M. (1985) Topographic localization of calcitonin gene-related peptide in the rat brain: an immunohistochemical analysis. *Neuroscience* **15,** 747–763.

Kemp B. E., Moseley J. M., Rodda C. P., Ebeling P. R., Wettenhall R. E. H., Stapleton D., Diefenbach-Jagger H., Ure F., Michelangeli V. P., Simmons H. A., Raisz L. G., and Martin T. J. (1987) Parathyroid hormone related protein of malignancy, active synthetic fragments. *Science* **23,** 1568–1570.

Krahn D. D., Gosnell B. A., Levine A. S., and Morley J. E. (1984) Effects of calcitonin gene-related peptide on food intake. *Peptides* **5,** 861–864.

Kruger L., Mantyh P. W., Sternini C., Brecha N. C., and Mantyh C. R. (1988) Calcitonin gene-related peptide (CGRP) in the rat central nervous system: patterns of immunoreactivity and receptor binding sites. *Brain Res.* **463,** 223–244.

Lasmoles F., Jullienne A., Day F., Minvielle S., Milhaud G., and Moukhtar M. S. (1985) Elucidation of the nucleotide sequence of chicken calcitonin mRNA: direct evidence for the expression of a lower vertebrate calcitonin-like gene in man and rat. *EMBO J.* **4,** 2603–2607.

Lengyel A. J. and Tannenbaum G. S. (1987) Mechanisms of calcitonin-induced growth hormone (GH) suppression: roles of somatostatin and GH-releasing factor. *Endocrinology* **120**, 1377–1383.

Lenz H. J. (1988) Calcitonin and CGRP inhibit gastrointestinal transit via distinct neuronal pathways. *Am. J. Physiol.* **254**, G920–G924.

Lenz H. J. and Brown M. R. (1990) Cerebroventricular calcitonin gene-related peptide inhibits rat duodenal bicarbonate secretion by release of norepinephrine and vasopressin. *J. Clin. Invest.* **85**, 25–32.

Lenz H. J., Mortrud M. T., Rivier J. E., and Brown M. R. (1985) Central nervous system actions of calcitonin gene-related peptide on gastric acid secretion in the rat. *Gastroenterology* **88**, 539–544.

Lenz H. J., Klapdor R., Hester S. E., Webb V. J., Galyean R. F., Rivier J. E., and Brown M. R. (1986) Inhibition of gastric acid secretion by brain peptides in the dog. Role of the autonomic nervous system and gastrin. *Gastroenterology* **91**, 905–912.

Lin H. Y., Harris T. L., Flannery M. S., Aruffo A., Kaji E. H., Gorn A., Kolakowski L. F. Jr., Lodish H. F., and Goldring S. R. (1991) Expression cloning of an adenylate cyclase-coupled calcitonin receptor. *Science* **254**, 1022–1024.

Lips C. J. M., Steenbergh P. H., Hoppener J. W. M., Bovenberg R. A. L., van der Sluys Veer J., and Jansz H. S. (1988) Evolutionary pathways of the calcitonin genes. *Mol. Cell. Endocrinol.* **57**, 1–6.

MacInnes D. G., Laszlo I., MacIntyre I., and Fink G. (1982) Salmon calcitonin in lizard brain: a possible neuroendocrine transmitter. *Brain Res.* **251**, 371–373.

Martin T. J., Ng K. W., and Nicholson G. C. (1988) Cell biology of bone, in *Clinical Endocrinology and Metabolism. Metabolic Bone Disease*, vol. 2, Martin T. J., ed., Bailliere Tindall, London, pp. 1–30.

Meadows R. P., Nikonowicz E. P., Jones C. R., Bastian J. W., and Gorenstein D. G. (1991) Two-dimensional NMR and structure determination of salmon calcitonin in methanol. *Biochemistry* **30**, 1247–1254.

Miyauchi T., Sano Y., Hiroshima O., Yuzuriha T., Sugishita Y., Ishikawa T., Saito A., and Goto K. (1988) Positive inotropic effects and receptors of calcitonin gene-related peptide (CGRP) in porcine ventricular muscles. *Biochem. Biophys. Res. Commun.* **155**, 289–294.

Morimoto T., Okamoto M., Koida M., Nakamuta H., Stahl G. L., and Orlowski R. C. (1985) Intracerebroventricular injection of ^{125}I-salmon calcitonin in rats: fate, anorexia and hypocalcemia. *Japan J. Pharmacol.* **37**, 21–29.

Morley J. E., Levine A. S., and Silvis S. E. (1981) Intraventricular calcitonin inhibits gastric acid secretion. *Science* **214**, 671–673.

Morton C. R., Maisch B., and Zimmermann M. (1986) Calcitonin brainstem microinjection but not systemic administration inhibits spinal nociceptive transmission in the cat. *Brain Res.* **372**, 149–154.

Nakamuta H., Orlowski R. C., and Epand R. M. (1990) Evidence for calcitonin receptor heterogeneity: binding studies with nonhelical analogs. *Endocrinology* **127**, 163–169.

Nakamuta H., Furukawa S., Koida M., Yajima H., Orlowski R. C., and Schlueter R. (1981) Specific binding of ^{125}I-salmon CT to rat brain: regional variation and calcitonin specificity. *Japan. J. Pharmacol.* **31**, 53–60.

Niall H. D. (1982) The evolution of peptide hormones. *Ann. Rev. Physiol.* **44**, 615–624.

Nicholson G. C., Moseley J. M., Sexton P. M., and Martin T. J. (1987) Characterization of calcitonin receptors and cyclic AMP responses in isolated osteoclasts. *Calcium and Bone Metabolism: Basic and Clinical Aspects, vol. 9.* Cohn D. V., Martin T. J., and Meunier P. J., eds., Elsevier Science Publishers, Amsterdam, pp. 343–348.

Nicholson G. C., Moseley J. M., Sexton P. M., Mendelsohn F. A. O., and Martin T. J. (1986) Abundant calcitonin receptors in isolated rat osteoclasts: biochemical and autoradiographic characterization. *J. Clin. Invest.* **78**, 355–360.

Nicosia S., Guidobono F., Musanti M., and Pecile A. (1986) Inhibitory effects of calcitonin on adenylate cyclase activity in different rat brain areas. *Life Sci.* **39**, 2253–2262.

Nicoletti F., Clementi G., Patti F., Canonico P. L., Di Gorgio R. M., Matera M., Pennisi G., Angelucci L., and Scapagnini U. (1982) Effects of calcitonin on rat extrapyramidal motor system. Behavioural and biochemical data. *Brain Res.* **250**, 381–385.

Olgiati V. R., Guidobono F., Netti C., and Pecile A. (1983) Localization of calcitonin binding sites in rat central nervous system: evidence of its neuroactivity. *Brain Res.* **265**, 209–215.

Olgiati V. R., Guidobono F., Luisetto G., Netti C., Biandni C., and Pecile A. (1981) Calcitonin inhibition of physiological and stimulated prolactin secretion in rats. *Life Sci.* **29**, 585–594.

Orlowski R. C., Epand R. M., and Stafford A. R. (1987) Biologically potent analogues of salmon CT which do not contain an N-terminal disulfide-bridged structure. *Eur. J. Biochem.* **162**, 399–402.

Patel J., Fabbri A., Pert C., Gnessi L., Fraioli F., and McDevitt R. (1985) Calcitonin inhibits the phosphorylation of various proteins in rat brain synaptic membranes. *Biochem. Biophys. Res. Commun.* **130,** 669–676.

Perez Cano R., Girgis S. I., and MacIntyre I. (1982a) Further evidence for calcitonin gene duplication: the identification of two different calcitonins in a fish, a reptile and two mammals. *Acta Endocrinol.* **100,** 256–261.

Perez Cano R., Girgis S. I., Galan Galan F., and MacIntyre I. (1982b) Identification of both human and salmon calcitonin-like molecules in birds suggesting the existence of two calcitonin genes. *J. Endocrinol.* **92,** 351–355.

Perez Cano R., Galan Galan F., Girgis S. I., Arnett T. R., and MacIntyre I. (1981) A human calcitonin-like molecule in the ultimobranchial body of the amphibia (Rang pipiens). *Experentia* **37,** 1116,1117.

Plata-Salaman C. R. and Oomura Y. (1987) Calcitonin as a feeding suppressant: localization of central action to cerebral III ventricle. *Physiol. Behav.* **40,** 501–513.

Prieto G. J., Cannon J. T., and Leibeskind J. C. (1983) Raphe magnus lesions disrupt stimulation produced analgesia from ventral but not dorsal midbrain areas in the rat. *Brain Res.* **261,** 53–57.

Rapisarda E., Clementi G., Fiore L., Prato A., Ceravolo A., Raffaele R., and Scapagnini U. (1984) Effect of calcitonin on ACTH secretion. *Pharmacol. Res. Commun.* **16,** 1151–1159.

Rizzo A.J. and Goltzman D. (1981) Calcitonin receptors in the central nervous system of the rat. *Endocrinology* **108,** 1672–1677.

Rodrigo J., Polak J. M., Terenghi G., Cervantes C., Ghatei M. A., Mulderry P. K., and Bloom S. R. (1985) Calcitonin gene-related peptide (CGRP)-immunoreactive sensory and motor nerves of the mammalian palate. *Histochemistry* **82,** 67–74.

Rosenfeld M. G., Mermod J.-J., Amara S. G., Swanson L. W., Sawchenko P. E., Rivier J., Vale W. W., and Evans R. M. (1983) Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. *Nature* **304,** 129–135.

Sabbatini F., Fimmel C. J., Pace F., Tobler P. H., Hinder R. A., Blum A. L., and Fischer J. A. (1985) Distribution of intraventricular salmon calcitonin and suppression of gastric acid secretion. *Digestion* **32,** 273–281.

Sagar S. M., Henke H., and Fischer J. A. (1984) Calcitonin and calcitonin gene-related peptide in the human brain. *Psychopharmacol. Bull.* **20,** 447–450.

Sano Y., Hiroshima O., Yuzuriha T., Yamato C., Saito A., Kimura S., Hirabayashi T., and Goto K. (1989) Calcitonin gene-related peptide-binding sites of porcine cardiac muscles and coronary arteries: solubilization and characterization. *J. Neurochem.* **52,** 1919–1924.

Seifert H., Chesnut J., de Souza E., Rivier J., and Vale W. W. (1985) Binding sites for calcitonin gene-related peptide in distinct areas of rat brain. *Brain Res.* **346,** 195–198.

Sexton P. M., McKenzie J. S., and Mendelsohn F. A. O. (1988) Evidence for a new subclass of calcitonin/calcitonin gene-related peptide binding site in rat brain. *Neurochem. Int.* **12,** 323–335.

Sexton P. M., Adam W. R., Moseley J. M., Martin T. J., and Mendelsohn F. A. O. (1987) Localization and characterization of renal calcitonin receptors by *in vitro* autoradiography. *Kidney Int.* **32,** 862–868.

Sexton P. M., McKenzie J. S., Mason R. T., Moseley J. M., Martin T. J., and Mendelsohn F. A. O. (1986) Localization of binding sites for calcitonin gene-related peptide by *in vitro* autoradiography. *Neuroscience* **19,** 1235–1245.

Sexton P. M., D'Santos C. S., Mendelsohn F. A. O., Schneider H.-G., Findlay D. M., Kemp B. E., and Moseley J. M. (1990) Characterization of sheep brain calcitonin receptors. *Calcium Regulation and Bone Metabolism. Basic and Clinical Aspects, vol. 10.* Cohn D. V., Glorieux F. H., and Martin T. J., eds., Elsevier Science Publishers, Amsterdam, pp. 57–63.

Sexton P. M., Schneider H.-G., D'Santos C. S., Mendelsohn F. A. O., Kemp B. E., Moseley J. M., Martin T. J., and Findlay D. M. (1991) Reversible calcitonin binding to solubilized sheep brain binding sites. *Biochem. J.* **273,** 179–184.

Shimizu N. and Oomura Y. (1986) Calcitonin-induced anorexia in rats: evidence for its inhibitory action on lateral hypothalamic chemosensitive neurons. *Brain Res.* **367,** 128–140.

Skofitsch G. and Jacobowitz D. M. (1985a) Autoradiographic distribution of ^{125}I calcitonin gene-related peptide binding sites in the rat central nervous system. *Peptides* **6,** 975–986.

Skofitsch G. and Jacobowitz D. M. (1985b) Calcitonin gene-related peptide: detailed immunohistochemical distribution in the rat central nervous system. *Peptides* **6,** 721–745.

Steenbergh P. H., Hoppener J. W. M., Zandberg J., Lips C. J. M., and Jansz H. S. (1985) A second human calcitonin/CGRP gene. *FEBS Lett.* **183,** 403–407.

Tache Y., Gunion M., Lauffenberger M., and Goto Y. (1984) Inhibition of gastric acid secretion by intracerebral injection of calcitonin gene-related peptide in rats. *Life Sci.* **35,** 871–878.

Takami K., Kawai Y., Shiosaka S., Lee Y., Girgis S. I., Hillyard C. J., MacIntyre I., Emson P. C., and Tohyama M. (1985a) Immunohistochemical evidence for the existence of calcitonin gene-related peptide—and choline acetyltransferase-like immunoreactivity in neurons of the rat hypoglossal, facial and ambiguus nuclei. *Brain Res.* **328,** 386–389.

Takami K., Kawai Y., Uchida S., Tohyama M., Shiotani Y., Yoshida H., Emson P. C., Girgis S. I., Hillyard C. J., and MacIntyre I. (1985b) Effect of calcitonin gene-related peptide on contraction of striated muscle in the mouse. *Neurosci. Lett.* **60,** 227–230.

Tam J. P., Marquardt H., Rosberger D. F., Wong T. W., and Todaro G. J. (1984) Synthesis of biologically active rat transforming growth factor 1. *Nature* **309,** 376–378.

Tannenbaum G. S. and Goltzman D. (1985) Calcitonin gene-related peptide mimics calcitonin actions in brain on growth hormone release and feeding. *Endocrinology* **116,** 2685–2687.

Tschopp F. A., Henke H., Petermann J. B., Tobler P. H., Janzer R., Hokfelt T., Lundberg J. M., Cuello C., and Fischer J. A. (1985) Calcitonin gene-related peptide and its binding sites in the human central nervous system and pituitary. *Proc. Natl. Acad. Sci. USA* **82,** 248–252.

Twery M. J., Kirkpatrick B., Lewis M. H., Mailman R. B., and Cooper C. W. (1986a) Antagonistic behavioral effects of calcitonin and amphetamine in the rat. *Pharmacol. Biochem. Behav.* **24,** 1203–1207.

Twery M. J., Kirkpatrick B., Critcher E. C., Lewis M. H., Mailman R. B., and Cooper C. W. (1986b) Motor effects of calcitonin administered intracerebroventricularly in the rat. *Eur. J. Pharmacol.* **121,** 189–198.

Twery M. J., Seitz P. K., Nickols G. A., Cooper C. W., Gallagher J. P., and Orlowski R. C. (1988) Analogue separates biological effects of salmon calcitonin on brain and renal cortical membranes. *Eur. J. Pharmacol.* **155,** 285–292.

Vallejo M., Lightman S., and Marshall I. (1988) Central cardiovascular effects of calcitonin gene-related peptide: interaction with noradrenaline in the nucleus tractus solitarius of rats. *Exp. Brain Res.* **70,** 221–224.

Van Houten M., Rizzo A. J., Goltzman D., and Posner B. I. (1982) Brain receptors for blood-borne calcitonin in rats: circumventricular localization and vasopressin-resistant deficiency in hereditary diabetes insipidus. *Endocrinology* **111,** 1704–1710.

Watahiki M., Yamamoto M., Yamakawa M, Tanaka M., and Nakashima K. (1989) Conserved and unique amino acid residues in the domains of the growth hormones. Flounder growth hormones deduced from the cDNA sequence has the minimal size in the growth hormone prolactin gene family. *J. Biol. Chem.* **264,** 312–316.

Welch S. P., Cooper C. W., and Dewey W. L. (1986) Antinociceptive activity of salmon calcitonin injected intraventricularly in mice: modulation of morphine antinociception. *J. Pharmacol. Exp. Ther.* **237,** 54–58.

Yoshizaki H., Takamiya M., and Okada T. (1987) Characterization of picomolar affinity binding sites for [^{125}I]human calcitonin gene-related peptide in rat brain and heart. *Biochem. Biophys. Res. Commun.* **146,** 443–451.

Zaidi M., Moogna B. S., Bevis P. S., Bascal Z. A., and Breimer L. H. (1990) The calcitonin gene peptides: biology and clinical relevance. *Crit. Rev. Clin. Lab. Sci.* **28,** 109–174.