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Distribution of N-type Ca^{2+} channel binding sites in rabbit brain following central administration of ω-conotoxin GVIA

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Abstract

Central administration of the N-type Ca^{2+} channel blocker ω-conotoxin GVIA in conscious rabbits has previously been shown to result in a slowly developing hypotensive and sympatholytic effect, with peak changes observed after 48 h. The aim of the current study was to examine the distribution of [^{125}I]ω-conotoxin GVIA binding in rabbit brain alone or following a prior i.c.v. injection of ω-conotoxin GVIA to determine the site(s) of action of centrally administered ω-conotoxin GVIA. Brains were removed from rabbits 2 or 48 h after central administration of vehicle or non-labelled ω-conotoxin GVIA (30 pmol/kg, i.c.v.). Brain sections were then incubated with [^{125}I]ω-conotoxin GVIA (50 pM) and the density of specific [^{125}I]ω-conotoxin GVIA binding measured in dpm/mm^2 was determined by quantitative receptor autoradiography. In the vehicle group, highest densities of [^{125}I]ω-conotoxin GVIA binding sites (> 20 dpm/mm^2) were detected in cortex, caudate, putamen, and the stratum oriens and stratum radiatum of the hippocampus. Prior (48 h) i.c.v. injection of ω-conotoxin GVIA resulted in a decrease in specific binding of [^{125}I]ω-conotoxin GVIA, particularly in cortex and some portions of the caudate and hippocampus. Lesser effects were observed with a prior (2 h) i.c.v. injection of ω-conotoxin GVIA. Central administration of ω-conotoxin GVIA may be acting to disrupt neurotransmission in higher brain regions which may, in turn, affect cardiovascular control mechanisms in the rabbit.

Keywords: Autoradiography; Brain; ω-Conotoxin GVIA; Ca^{2+} channel, N-type ; (Rabbit)

1. Introduction

A number of classes of voltage-operated Ca^{2+} channel (VOCC) have been identified in the central nervous system and periphery. These include L-, N-, P- and the more recently identified Q-type voltage-operated Ca^{2+} channels (Olivera et al., 1994). Various channel types are involved in the central regulation of neurotransmitter release (Dooley et al., 1987; Luebke et al., 1993; Wheeler et al., 1994). N-type voltage-operated Ca^{2+} channels, located only on neuronal tissue (McCleskey et al., 1987), are selectively blocked by ω-conotoxin GVIA, a 27 amino acid peptide isolated from the venom of the cone snail *Conus geographus* (Hirning et al., 1988; Olivera et al., 1984).

Previous studies have utilised [^{125}I]ω-conotoxin GVIA as a tool to determine the binding characteristics and localisation of binding sites for ω-conotoxin GVIA. Binding and autoradiographic studies have been performed in a number of species including rat, mouse, dog and human (Albensi et al., 1993; Kerr et al., 1988; Perry et al., 1994; Takemura et al., 1989). These papers describe a heterogeneous distribution of [^{125}I]ω-conotoxin GVIA binding sites in the brain with high levels of binding in cortex, hippocampus and caudate, as well as areas important in cardiovascular control such as the nucleus of the solitary tract. Some differences in specific [^{125}I]ω-conotoxin GVIA binding have also been observed during different stages of brain development (Dooley et al., 1988; Filloux et al., 1994b). The distribution of binding sites for [^{125}I]ω-conotoxin GVIA differs from L-type Ca^{2+} channel blockers (Dooley et al., 1988; Perry et al., 1994) and ω-conotoxin MVIIC (Filloux et al., 1994a) which targets P- and Q-, as well as N-type, voltage-operated Ca^{2+} channels (Wright and Angus, 1996). While the N-type VOCC has previously been purified and cloned from rabbit brain (Fujita et al., 1993), the distribution of binding sites for [^{125}I]ω-conotoxin GVIA in rabbit brain has not yet been determined.

Earlier work in our laboratory examined the cardiovascular effects of lateral ventricle (i.c.v.) administration

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of ω-conotoxin GVIA in the conscious rabbit (Whorlow et al., 1994). Central administration of ω-conotoxin GVIA (30 pmol/kg i.c.v.) resulted in a fall in mean arterial pressure and an attenuation of the sympathetic, but not vagal, component of the baroreceptor-heart rate reflex (baroreflex). The time course for the development of this sympatholytic activity was slow and similar to that for the hypotension, taking 48 h to plateau. In order to investigate the basis for this long time course of action of ω-conotoxin GVIA, we have used quantitative autoradiography to examine the distribution of binding sites for [^{125}I]ω-conotoxin GVIA in rabbit brain following i.c.v. administration of ω-conotoxin GVIA.

2. Materials and methods

2.1. Surgical procedures

New Zealand white rabbits of either sex (2.42 ± 0.03 kg, $n = 10$) were used in the study. Lateral ventricle cannulae were implanted under halothane anaesthesia (Fluothane, ICI, Melbourne, Australia) following induction with i.v. Saffan (alphaxalone/alphadolone; Pitman-Moore, Sydney, Australia) as described previously (Whorlow et al., 1994). Correct placement of the guide cannula was tested by inserting the injection cannula attached to tubing filled with saline and allowing flow under hydrostatic pressure. The guide cannula was then sealed with a dummy cannula and rabbits were allowed to recover for 7–10 days before experiments commenced. In all cases, cannula placement was confirmed histologically when brains were sectioned.

On each experimental day, the central ear artery and marginal ear vein were cannulated under local anaesthesia (0.5% lignocaine hydrochloride; Xylocaine, Astra, Sydney, Australia). The ear artery catheter was connected to a CDX pressure transducer (Cobe, Lakewood, CO, USA) for the measurement of phasic and mean arterial pressure which was recorded on a Grass polygraph (Model 7D, Quincy, MA, USA). A rate meter (Model 173, Baker Medical Research Institute, Melbourne, Australia) was triggered by the phasic arterial pressure for the measurement of heart rate.

2.2. In vivo experiments

Experiments were performed over 1 or 3 days following a similar protocol to that described previously (Whorlow et al., 1994). On Day 1 (0 h), the baroreceptor-heart rate reflex (baroreflex) curve was performed by inducing graded changes in mean arterial pressure (±5–30 mmHg from baseline) with intravenous injections of phenylephrine and glyceryl trinitrate (Head and McCarty, 1987). ω-Conotoxin GVIA (30 pmol/kg in 10 μl, $n = 5$) or vehicle (10 μl saline, $n = 5$) was then administered as an i.c.v. bolus over about 60 s and resting mean arterial pressure and heart rate were monitored for 2 h. The baroreflex curve was then retested. At the completion of Day 1, 2 rabbits from each of the ω-conotoxin GVIA and vehicle groups were perfused and brains removed as described below. On days 2–3 (24–48 h) the baroreflex curve was performed in the remaining rabbits, however no further ω-conotoxin GVIA was administered. At the completion of Day 3, rabbits were perfused as described below. Between experimental days, animals were returned to their home cages and their behaviour closely monitored.

At the completion of the experimental period, rabbits were injected with heparin (1000 U i.v.; David Bull, Melbourne, Australia) then deeply anaesthetised with i.v. Saffan. The carotid arteries were isolated and cannulated and the jugular veins cut to allow drainage of the perfusate. Rabbits were then given an overdose of i.v. pentobarbitone (Nembutal, Boehringer Ingelheim, Australia). Carotid arteries were perfused with phosphate-buffered saline (pH 7.4) for 10 min at 90–100 mmHg, then with phosphate-buffered saline containing 0.5% paraformaldehyde for a further 10 min at 90–100 mmHg. Prefixing brains with paraformaldehyde does not affect the distribution of [^{125}I]ω-conotoxin GVIA binding sites (Takemura et al., 1989). Brains were rapidly removed, frozen over liquid nitrogen and stored at −70°C.

2.3. Autoradiography

Brains were mounted on brass chucks with Tissue-Tek O.C.T. compound (Miles, Elkhart, IN, USA) in a Reichert-Jung cryostat at −17°C. Serial coronal sections 10 μm thick and located 3 mm rostral and 0.6, 4.6, 8.6, 12.6, 32.3 and 33.6 mm caudal to the site of i.c.v. injection were cut and mounted onto gelatin-chrome alum-coated slides. Regions of brain were identified according to the atlas of Shek et al. (1986). Slide-mounted sections were stored at −70°C in boxes containing silica gel.

Prior to labelling with [^{125}I]ω-conotoxin GVIA, slide-mounted sections were allowed to thaw to room temperature. Consecutive slides were selected for total and non-specific binding with every third slide stored for later staining with cresyl violet (0.1% thionin acetate, Sigma, St. Louis, MO, USA) for histological examination. The protocol for autoradiography was adapted from that of Takemura et al. (1989). Duplicate sections were preincubated for 10 min at 4°C in a buffer containing sucrose (0.32 mM), bovine serum albumin (1 mg/ml) and Hepes (5 mM, pH 7.4). Sections were then incubated for 90 min at 4°C in the same buffer as above containing [^{125}I]ω-conotoxin GVIA (50 pM). Non-specific binding was determined by the addition of 1 μM ω-conotoxin GVIA. Sections underwent 6 washes of 10 min each at 4°C in a buffer consisting of choline chloride (160 mM), $CaCl_2$ (1.5 mM), bovine serum albumin (1 mg/ml) and Tris (pH 7.4) with a final dip in distilled water and were air dried and

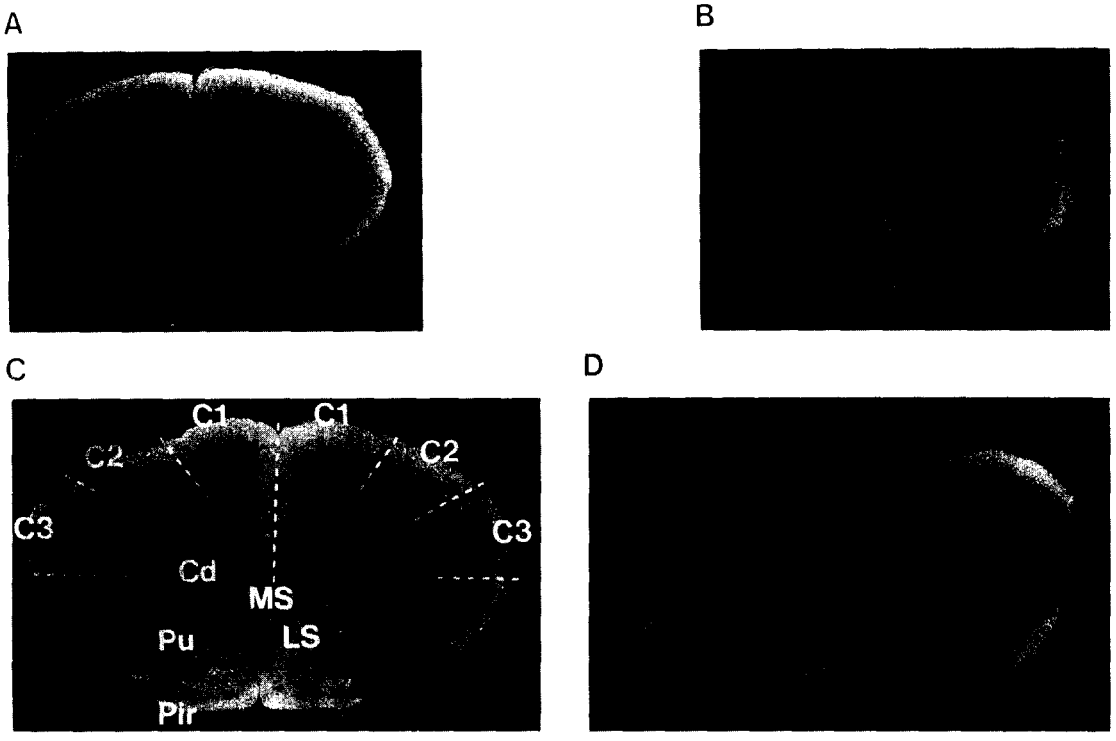

Fig. 1. Autoradiograms of total binding of [^{125}I]ω-conotoxin GVIA in rabbit brain. Sections were obtained from rabbits 48 h after i.c.v. injection of vehicle (A and C) or 30 pmol/kg ω-conotoxin GVIA (B and D) on the left side of the brain. Relative to the site of injection, sections were located 3 mm rostral (A and B) or 0.6 mm caudal (C and D). The lightest areas indicate the highest density of [^{125}I]ω-conotoxin GVIA binding sites. C1–3, cortex regions 1–3 as defined in Materials and methods.

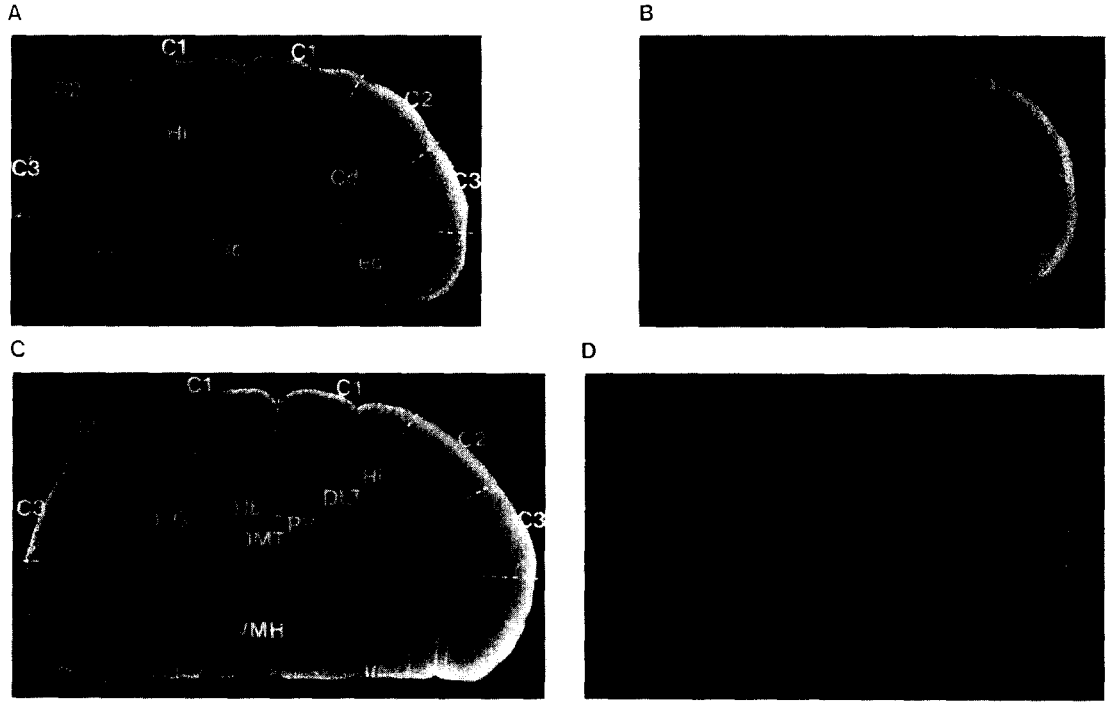

Fig. 2. Autoradiograms of total binding of [^{125}I]ω-conotoxin GVIA in rabbit brain. Sections were obtained from rabbits 48 h after i.c.v. injection of vehicle (A and C) or 30 pmol/kg ω-conotoxin GVIA (B and D) on the left side of the brain. Relative to the site of injection, sections were located 4.6 (A and B) or 8.6 mm caudal (C and D). The lightest areas indicate the highest density of [^{125}I]ω-conotoxin GVIA binding sites. C1–3, cortex regions 1–3 as defined in Materials and methods. Hi, hippocampus.

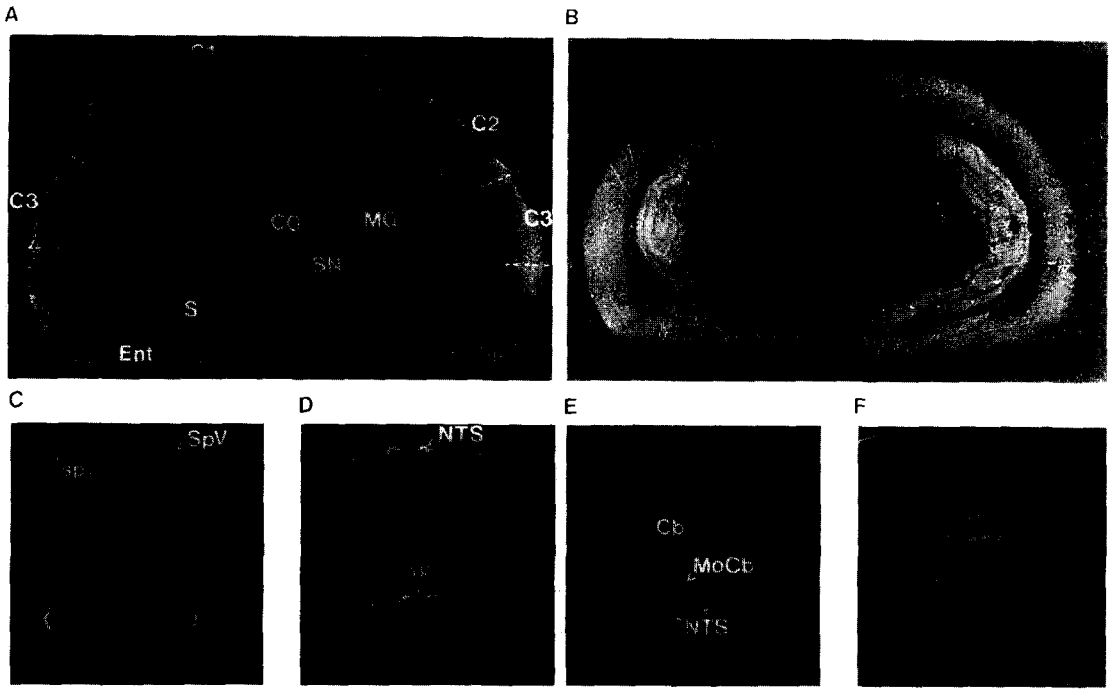

Fig. 3. Autoradiograms of total binding of [^{125}I]ω-conotoxin GVIA in rabbit brain. Sections were obtained from rabbits 48 h after i.c.v. injection of vehicle (A, C and E) or 30 pmol/kg ω-conotoxin GVIA (B, D and F) on the left side of the brain. Relative to the site of injection, sections were located 12.6 (A and B), 33.6 (C and D) or 32.3 mm caudal (E and F). The lightest areas indicate the highest density of [^{125}I]ω-conotoxin GVIA binding sites. C1–3, cortex regions 1–3 as defined in Materials and methods. Cb, cerebellum.

apposed to film (Hyperfilm βmax, Amersham) along with ^{125}I rat brain paste standards. After 7–10 days exposed in X-ray cassettes at −20°C, the film was developed (Kodak D19) and fixed (Kodak rapid fixer). ω-Conotoxin GVIA dissociates very slowly from its binding site (Cruz and Olivera, 1986), so prior i.c.v. administration would presumably cause an exclusion of subsequent [^{125}I]ω-conotoxin GVIA binding at the site(s) of action of ω-conotoxin GVIA.

2.4. Data analysis and statistics

Data are expressed as means ± 1 standard error of the mean (S.E.M.) where appropriate. The mean arterial pressure and heart rate responses to phenylephrine and glyceryl trinitrate were measured as peak changes. Mean arterial pressure and heart rate changes obtained for the baroreflex were fitted to a sigmoidal logistic equation (Head and McCarty, 1987). Values for lower heart rate plateau (beats/min), heart rate range (beats/min) between upper and lower plateaus of the curve and median blood pressure (MAP_{50} (mmHg)) were analysed to characterise the baroreflex curves.

Density of [^{125}I]ω-conotoxin GVIA specific binding sites was determined by comparison with ^{125}I brain paste standards using a Micro-Computer Imaging Device (MCID-M1, Imaging Research, Ontario, Canada). Density of binding sites was calculated as dpm/mm^2 with non-specific binding subtracted from total binding to determine specific binding. To determine the extent of [^{125}I]ω-conotoxin GVIA binding throughout the cortex, regions were sub-divided into several sections (see Fig. 1 and Fig. 2 and Fig. 3) and defined as cortex regions 1–3 (C1–3). Comparisons were made between vehicle- and ω-conotoxin GVIA pretreatment groups at 2 h and 48 h time points by Student's *t*-test for unpaired data. Probability values less than 0.05 were accepted as statistically significant.

2.5. Drugs

Drugs used and their suppliers were: ω-conotoxin GVIA (synthesised by J.P. Flinn (Flinn et al., 1995), Department of Pharmacology, University of Melbourne, Australia), [^{125}I]Tyr^{22}-ω-conotoxin GVIA (DuPont NEN, Boston, MA, USA), glyceryl trinitrate (David Bull Laboratories, Melbourne, Australia) and L-phenylephrine hydrochloride (Sigma). Glyceryl trinitrate and phenylephrine were diluted in 0.9% NaCl solution (saline). ω-Conotoxin GVIA stock solution (10^{-4} M) was stored at −20°C and diluted in saline as required. The stock solution of [^{125}I]ω-conotoxin GVIA was diluted in distilled water and stored at −20°C.

3. Results

3.1. In vivo experiments

No changes in cardiovascular parameters, nor the baroreflex, were observed in the vehicle-treated groups ($n = 2–3$) or in the 2 h ω-conotoxin GVIA-treated group ($n = 2$). In the 48-h-ω-conotoxin GVIA-treated animals,

mean arterial pressure decreased from 70 ± 5 mmHg at 0 h to 62 ± 2 mmHg 48 h after i.c.v. administration of ω-conotoxin GVIA ($n = 3$), with no change in heart rate from the 0 h control value of 172 ± 8 beats/min. A leftward shift in the baroreflex curve was seen with a decrease in the MAP_{50} from 61 ± 4 to 54 ± 2 mmHg between 0 and 48 h. There was no change in the lower heart rate plateau of the baroreflex from 135 ± 4 beats/min at 0 h, however there was a trend for a decrease in the heart rate range of the curve indicating a sympatholytic action of ω-conotoxin GVIA as described previously (Whorlow et al., 1994).

3.2. Binding sites for [^{125}I]ω-conotoxin GVIA in rabbit brain

Specific binding sites for [^{125}I]ω-conotoxin GVIA were distributed throughout rabbit brain. In the forebrain (Fig. 1 and Fig. 2 and Fig. 3; Table 1) highest densities (specific binding > 20 dpm/mm^2) of [^{125}I]ω-conotoxin GVIA binding sites were observed in all cortical regions, caudate and putamen. More intermediate densities of binding (10–20 dpm/mm^2) were observed in the lateral and medial septum, thalamic nuclei, ventromedial hypothalamus, medial geniculate nucleus and substantia nigra. Low densities (< 10 dpm/mm^2) were observed in the anterior commissure, habenular nucleus, dorsolateral geniculate nucleus and central grey. In the hippocampus (Fig. 2), high densities of [^{125}I]ω-conotoxin GVIA binding were observed in the stratum oriens and stratum radiatum with intermediate levels in the molecular and granule cell layers. No specific binding was observed in the pyramidal cell layer. In the hindbrain (Fig. 3, C–F), lower densities of [^{125}I]ω-conotoxin GVIA binding were detected than in more rostral regions, with binding not exceeding 10 dpm/mm^2 in any area. Specific [^{125}I]ω-conotoxin GVIA binding was ob-

Table 1
Specific binding of [^{125}I]ω-conotoxin GVIA determined by autoradiographic analysis in rabbit brains 48 h following administration of vehicle or 30 pmol/kg ω-conotoxin GVIA (i.c.v.)

Region	Abbr.	Veh L	Veh R	ω-CTX L	ω-CTX R
Anterior commissure [c]	ac	5.0 ± 1.9 [a]	5.0 ± 1.9 [a]	2.8 ± 1.1	2.8 ± 1.1
Area postrema [g]	AP	4.7 ± 1.2	4.7 ± 1.2	4.2 ± 0.8	4.2 ± 0.8
Caudate [b]	Cd	20.2 ± 4.9	19.4 ± 3.7	15.0 ± 1.9	19.1 ± 2.4
Caudate [c]	Cd	23.4 ± 5.7	24.1 ± 5.8	12.9 ± 1.0	15.3 ± 3.4
Central grey [c]	CG	6.3 ± 0.9	6.3 ± 0.9	4.1 ± 1.9	4.1 ± 1.9
Dorsolateral geniculate nucleus [d]	DLG	8.0 ± 2.4	7.2 ± 1.9	6.7 ± 0.4	5.5 ± 1.2
Dorsolateral thalamic nucleus [d]	DLT	13.5 ± 3.0	13.4 ± 2.6	13.1 ± 0.9	11.4 ± 1.7
Dorsomedial thalamic nucleus [d]	DMT	10.3 ± 2.2	11.6 ± 2.8	12.0 ± 1.5	11.3 ± 0.9
Entorhinal Cortex [c]	Ent	20.8 ± 5.7	21.1 ± 5.9	17.6 ± 3.5	17.0 ± 1.9
External capsule [c]	ec	7.3 ± 2.2	10.5 ± 3.9	5.0 ± 1.2	6.4 ± 1.7
Granule cell layer of cerebellum [f]	GrCb	1.4 ± 0.3	1.4 ± 0.3	2.2 ± 0.6	2.2 ± 0.6
Granule cell layer of hippocampus [d]	GrHi	18.7 ± 3.8	18.6 ± 3.3	17.8 ± 1.9	17.0 ± 2.7
Habenular nucleus [d]	Hb	4.3 ± 0.9	5.1 ± 1.0	4.6 ± 1.3	4.5 ± 1.8
Internal capsule [c]	ic	4.5 ± 2.5	5.0 ± 1.6	2.5 ± 1.3	2.5 ± 0.7
Lateral septum [b]	LS	17.7 ± 2.7	17.7 ± 3.0	11.4 ± 4.3	14.7 ± 4.1
Medial geniculate nucleus [e]	MG	13.3 ± 3.6	12.5 ± 3.3	10.5 ± 3.0	12.0 ± 4.0
Medial septum [b]	MS	15.9 ± 3.2	15.9 ± 3.2	15.9 ± 4.3	15.9 ± 4.3
Molecular layer of cerebellum [f]	MoCb	4.5 ± 0.3	4.5 ± 0.3	5.7 ± 1.7	5.7 ± 1.7
Molecular layer of hippocampus [d]	MoHi	19.2 ± 4.5	19.9 ± 4.3	17.1 ± 2.9	16.5 ± 3.3
Nucleus of the solitary tract [f]	NTS	5.3 ± 0.6	5.0 ± 0.6	5.3 ± 1.1	4.8 ± 1.1
Nucleus of the solitary tract [g]	NTS	4.1 ± 1.0	4.1 ± 0.9	5.5 ± 1.2	5.5 ± 1.5
Paraventricular thalamic nucleus [d]	PV	13.3 ± 2.3	14.0 ± 2.8	14.1 ± 1.8	13.7 ± 2.2
Piriform cortex [b]	Pir	21.7 ± 5.6	16.0 ± 3.8	21.0 ± 7.9	18.1 ± 5.2
Putamen [b]	Pu	15.9 ± 2.8	14.9 ± 2.4	17.0 ± 3.9	17.1 ± 3.0
Putamen [c]	Pu	21.0 ± 5.0	21.1 ± 4.0	15.7 ± 2.3	16.6 ± 4.2
Spinal trigeminal nucleus [g]	SpV	6.0 ± 1.1	7.4 ± 2.1	6.9 ± 1.5	6.2 ± 1.9
Spinal trigeminal tract [g]	spv	1.1 ± 0.3	1.4 ± 0.6	1.8 ± 0.6	1.5 ± 0.4
Stratum oriens of hippocampus [d]	OrHi	20.0 ± 4.7	22.3 ± 4.6	16.0 ± 2.9	19.8 ± 4.1
Stratum radiatum of hippocampus [d]	RaHi	22.2 ± 5.2	24.0 ± 4.1	19.7 ± 3.8	20.8 ± 3.6
Subiculum [c]	S	18.4 ± 4.0	17.9 ± 4.9	15.9 ± 3.4	15.2 ± 2.5
Substantia nigra [e]	SN	13.7 ± 2.9	16.2 ± 5.3	7.3 ± 1.1	6.8 ± 1.5
Ventromedial hypothalamus [d]	VMH	18.4 ± 3.5	18.4 ± 3.5	14.6 ± 1.0	14.6 ± 1.0
White layer of cerebellum [f]	WhCb	0.8 ± 0.4	0.8 ± 0.4	1.4 ± 0.2	1.4 ± 0.2

Brains were removed 48 h after administration of vehicle (Veh, 10 μl saline, $n = 3$) or 30 pmol/kg ω-conotoxin GVIA (ω-CTX, $n = 3$) i.c.v. to conscious rabbits. Specific binding (dpm/mm^2) of [^{125}I]ω-conotoxin GVIA was determined by autoradiographic analysis. Densities were measured on both left (L, injection side) and right (R) sides of the brain where possible, or [a] a single value was obtained for medially located regions. Regions examined and abbreviations (Abbr.) were in sections located 0.6 [b], 4.6 [c], 8.6 [d], 12.6 [e], 32.3 [f] and 33.6 [g] mm caudal to the site of injection. Values are means ± 1 S.E.M.

served in the spinal trigeminal nucleus, nucleus of the solitary tract and area postrema. In the cerebellum, binding was detected in the molecular layer; no specific binding was observed in the granule cell layer.

3.3. Effect of ω-conotoxin GVIA i.c.v. on [^{125}I]ω-conotoxin GVIA binding

Similar patterns of [^{125}I]ω-conotoxin GVIA binding sites were observed in the 2 h and 48 h vehicle-treated brains ($n = 2$–3). The prior i.c.v. administration of unlabelled ω-conotoxin GVIA resulted in an inhibition of specific binding of [^{125}I]ω-conotoxin GVIA binding compared with time-matched vehicle-treated brain sections as determined by densitometric analysis. A smaller decrease of [^{125}I]ω-conotoxin GVIA binding was observed in brains removed from rabbits 2 h after i.c.v. ω-conotoxin GVIA, with the area of inhibition accounting for up to only 4% of the total area of the brain section ($n = 2$). In brains removed 48 h after i.c.v. injection of ω-conotoxin GVIA, the specific binding of [^{125}I]ω-conotoxin GVIA was inhibited by 20 and 16% of the total area of the section, 0.6 and 4.6 mm caudal to the site of injection, respectively (Figs. 1 and 2, $n = 3$). Histological examination of adjacent sections stained with cresyl violet revealed no neuronal damage in those areas where there was an absence of [^{125}I]ω-conotoxin GVIA binding (data not shown).

The extent of inhibition of specific [^{125}I]ω-conotoxin GVIA binding in brains removed from rabbits 48 h after i.c.v. administration of ω-conotoxin GVIA was most dramatic in frontal cortex in all sections examined, followed by the hippocampus and caudate (Figs. 1–3; Table 1). For densitometric analysis of the autoradiograms, the cortex was divided into 3 regions on each side of the brain (see Materials and methods and Figs. 1–3) to determine a more accurate picture of the inhibitory effect of ω-conotoxin GVIA. There was significantly less binding of [^{125}I]ω-conotoxin GVIA ($P < 0.05$, Fig. 4) in cortex regions 1 and 2 on the left (injection) side of the brain 0.6 mm caudal to the site of injection. There was also a slight reduction in [^{125}I]ω-conotoxin GVIA binding in the ω-conotoxin GVIA-treated brains in other sections examined. As described above, the most dramatic inhibitory effect on specific [^{125}I]ω-conotoxin GVIA binding in brains removed 48 h after i.c.v. administration of ω-conotoxin GVIA was observed in the sections located 0.6 and 4.6 mm caudal to the site of injection. The inhibition of [^{125}I]ω-conotoxin GVIA binding was not confined to the side of the brain in which ω-conotoxin GVIA was injected; an inhibitory effect of ω-conotoxin GVIA was also observed contralateral to the site of injection, with cortex being most affected (Figs. 1 and 2). There was a progressive decrease in the extent of inhibition caused by ω-conotoxin GVIA in sections more caudal to the site of injection. In the hindbrain, no inhibitory effect of ω-conotoxin GVIA was observed on [^{125}I]ω-conotoxin GVIA binding.

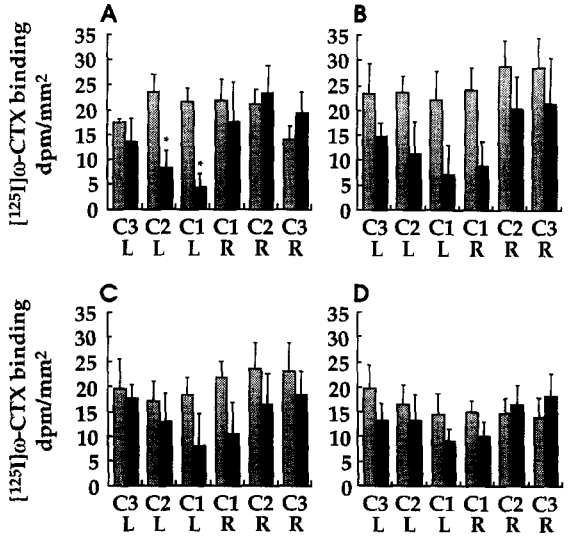

Fig. 4. Densities of [^{125}I]ω-conotoxin GVIA (specific binding) binding sites in rabbit cortex 48 h after i.c.v. injection of vehicle (stippled bars, $n = 3$) or 30 pmol/kg ω-conotoxin GVIA (black bars, $n = 3$) on the left side of the brain. Relative to the site of injection, sections were examined at 0.6 (A), 4.6 (B), 8.6 (C) and 12.6 mm (D) caudal. C1–3 are cortex regions 1–3 as defined in Methods on the left (L) and right (R) sides of the brain. See also Figs. 1–3. Error bars are ±1 S.E.M. *, $P < 0.05$, Student's t-test for unpaired data.

4. Discussion

[^{125}I]ω-Conotoxin GVIA binding sites are distributed throughout rabbit brain in a heterogeneous manner with a higher density located rostrally as has been described in rat brain (Kerr et al., 1988). Binding sites for [^{125}I]ω-conotoxin GVIA in the brain have been well characterised in a number of species, however this is the first description of the distribution of binding sites in rabbit brain. In general, there is a similar pattern of distribution between rabbit brain and other species. An exception is the observation of high densities of binding sites in the pyramidal cell layer of the hippocampus in human brain (Albensi et al., 1993); in the present study, and also previously reported in rat and dog brain (Perry et al., 1994; Takemura et al., 1989), very low densities of binding sites were observed in this region.

A number of subtypes of VOCC, including N-type, are involved in the central regulation of neurotransmitter release (Dooley et al., 1987; Wheeler et al., 1994). For example, in the hippocampus at least three Ca^{2+} channel subtypes are involved in release of neurotransmitter – N-, P- and Q-type channels – with little contribution of L-type Ca^{2+} channels (Luebke et al., 1993; Wheeler et al., 1994). Some overlap exists in the distribution pattern of binding sites, as determined autoradiographically, for ligands which target different voltage-operated Ca^{2+} channels. In dog brain, similar binding patterns for isradipine (an L-type

Ca^{2+} channel blocker) and ω-conotoxin GVIA were observed in cortex and striatum, but differences existed in hippocampus (Perry et al., 1994). Differences are also evident between binding sites for ω-conotoxin $MVIIC_{nle}$ (which blocks P- and Q-type channels) and ω-conotoxin GVIA in rat brain, particularly in cortex, cerebellum and thalamus (Filloux et al., 1994b).

Binding studies have determined that ω-conotoxin GVIA has a slow onset of action and dissociates very slowly (Cruz and Olivera, 1986). Following i.c.v. injection of ω-conotoxin GVIA, maximal changes in blood pressure and the baroreflex, indicative of sympatholysis, are seen after a period of 48 h (Whorlow et al., 1994). In the present study, the location of ω-conotoxin GVIA binding was examined autoradiographically at two time points (2 and 48 h) after i.c.v. administration by subsequent labelling with [^{125}I]ω-conotoxin GVIA. There was little distribution of ω-conotoxin GVIA throughout the brain 2 h after administration as comparable binding of [^{125}I]ω-conotoxin GVIA was seen in these and vehicle-treated sections. However, 48 h after administration of ω-conotoxin GVIA there was a substantial inhibition of [^{125}I]ω-conotoxin GVIA binding in the forebrain of the rabbits. There was no histological evidence of any neuronal damage caused by injection of ω-conotoxin GVIA, therefore such damage is an unlikely explanation for the lack of [^{125}I]ω-conotoxin GVIA binding. Further, in vehicle-treated rabbits, which had the same cannulae implanted, there was no disruption of [^{125}I]ω-conotoxin GVIA binding.

The relatively poor distribution of ω-conotoxin GVIA following i.c.v. injection was somewhat surprising. It has been established that cerebrospinal fluid is continually produced by the choroid plexus, and in rabbits, the rate of production is approximately 10 μl/min (Davson et al., 1987). In rabbits with normal intracranial pressure, injection of dye into the lateral ventricles resulted in the dye being distributed throughout the ventricular system within 4 h of administration (McComb et al., 1982). When intracranial pressure was elevated, dye could be observed in the episcleral tissue of the eyes after as little as 2 h and also the lymph nodes. In the present study ω-conotoxin GVIA should also spread throughout the ventricular system within approximately 4 h following i.c.v. injection. The slow distribution of ω-conotoxin GVIA following i.c.v. administration however does correlate with the slow onset of changes in mean arterial pressure and the baroreflex. It may be that ω-conotoxin GVIA is affecting cerebrospinal fluid formation or flow by an unknown mechanism. Previously in rabbits another peptide, angiotensin II, has been reported to have an inhibitory effect on cerebrospinal fluid production (Chodobski et al., 1992).

Binding sites for [^{125}I]ω-conotoxin GVIA had previously been described in the nucleus of the solitary tract (Takemura et al., 1989), which is the primary site of termination of baroreceptor afferent nerves (Dampney, 1994). Our hypothesis was that ω-conotoxin GVIA injected into the lateral ventricles would be able to flow through the ventricular system to exert its actions at this or other cardiovascular control centres. However the peptide remained localised around the area of injection – particularly in the cortex – suggesting that a region in the forebrain contributes to the slow time course observed for the hypotensive and sympatholytic effects of ω-conotoxin GVIA. Studies in rats have demonstrated that stimulation of areas of the insular cortex can modulate cardiovascular parameters. Moreover, projections from the insular cortex can be traced to other areas of the brain, including an area of the nucleus of the solitary tract (Yasui et al., 1991). Therefore, ω-conotoxin GVIA may be affecting neurotransmission at the level of the cortex rather than the medulla as first presumed.

It is of interest to our group that binding sites for [^{125}I]ω-conotoxin GVIA were detected in the area postrema. Following intravenous rather than central (i.c.v.) administration of ω-conotoxin GVIA, there is an attenuation of the vagally mediated, as well as sympathetically mediated, component of the baroreflex, without any effect on other reflexes mediated by the efferent vagus (Wright and Angus, 1995). Recently, it has been demonstrated that ω-conotoxin GVIA does not block current in putative aortic baroreceptor neurones (Cunningham et al., 1995). The area postrema is relatively free of a blood brain barrier and circulating peptides such as angiotensin can induce changes in blood pressure and the baroreflex via an action at neurones in this area (Dampney, 1994). There are also projections to other central cardiovascular control centres from the area postrema. Whether peripherally administered ω-conotoxin GVIA can influence central cardiovascular centres in this manner is yet to be determined.

In conclusion, the distribution of binding sites for [^{125}I]ω-conotoxin GVIA has been described for the first time in rabbit brain. In general, the pattern of binding determined autoradiographically is similar to that observed in other species. Administration of ω-conotoxin GVIA into the lateral ventricles of rabbits results in marked differences in the binding pattern of [^{125}I]ω-conotoxin GVIA, as around the site of injection binding of [^{125}I]ω-conotoxin GVIA is virtually eliminated. The distribution of ω-conotoxin GVIA was much wider in brains 48 h compared with 2 h after peptide injection. The changes in cardiovascular parameters observed 48 h after i.c.v. administration of ω-conotoxin GVIA may be due to disruption of cardiovascular pathways in the cortex.

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