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Serotonin-1A autoreceptor binding in the dorsal raphe nucleus of depressed suicides

Maura Boldrini[a,b], **Mark D. Underwood**[a], **J. John Mann**[a], and **Victoria Arango**[a,*]

a *Department of Psychiatry, Division of Neuroscience, Columbia University College of Physicians and Surgeons, New York State Psychiatric Institute, 1051 Riverside Drive, P.O. Box 42, New York, NY 10032, USA*

b *Department of Neurological and Psychiatric Sciences, University of Florence, Viale Morgagni 85, 50134 Firenze, Italy*

Abstract

Serotonergic dysfunction is present in mood disorders and suicide. Brainstem 5-HT_{1A} somatodendritic autoreceptors regulate serotonin neuron firing but studies of autoreceptor binding in the dorsal raphe nucleus (DRN) in depressed suicides report conflicting results. We sought to determine: (1) the anatomical distribution of 5-HT_{1A} receptor binding in the DRN in depressed suicides and psychiatrically normal controls; and (2) whether sex differences in 5-HT_{1A} binding in the DRN contribute to differences between depressed suicides and controls. Previously collected quantitative receptor autoradiograms of [^{3}H]8-hydroxy-2-(di-*n*-propyl)aminotetralin (^{3}H-8-OH-DPAT) in postmortem tissue sections containing the DRN from drug-free suicide victims ($n = 10$) and matched controls ($n = 10$) were analyzed. Less total receptor binding (fmol/mg tissue × mm^3) was observed in the entire DRN in depressed suicides compared with controls ($p < 0.05$). Group differences along the rostrocaudal extent of the DRN were observed for cross-sectional 5-HT_{1A} binding (fmol/mg tissue) and receptor binding (fmol/mg × mm^3, $p < 0.05$). Cross-sectional 5-HT_{1A} DRN binding in depressed suicides compared with controls was higher rostrally and lower caudally. The differences between depressed suicides and controls were present in males and females, although females had more binding than males. Less autoreceptor binding in the DRN of depressed suicides may represent a homeostatic response to less serotonin release, increasing serotonin neuron firing. More autoreceptor binding in rostral DRN might contribute to deficient serotonin release in ventromedial prefrontal cortex by lower neuronal firing.

Keywords

Serotonin; Suicide; 5-HT_{1A} autoreceptors; Postmortem; Dorsal raphe nucleus

1. Introduction

1.1. Background

Serotonergic abnormalities in major depressive disorder (MDD) and suicide include low serotonin (5-HT) and 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the brainstem, and fewer serotonin transporter (SERT) sites in cortex, hypothalamus and brainstem, suggesting less serotonin release in widespread areas of the brain (see, Mann, 2003 for review).

*Corresponding author. Tel.: +1 212 543 5440; fax: +1 212 543 6017. E-mail address: va19@columbia.edu (V. Arango).

Conflict of interest

All of the authors declare that they have no actual or potential conflicts of interest within three (3) years of beginning the work submitted that could inappropriately influence, or be perceived to influence, their work.

Suicide is distinguished from MDD by the localization of pre- and postsynaptic serotonergic changes. In suicide, there is less SERT and more 5-HT_{1A} binding in the ventral prefrontal cortex (PFC, Arango et al., 1995; Mann et al., 2000), a region involved in behavioral inhibition (Bechara et al., 2000). Using the 5-HT_{1A} antagonist 11-C-WAY100635, some studies report lower 5-HT_{1A} binding potential (BP) by positron emission tomography (PET) in the raphe and cortex in MDD (Drevets et al., 1999; Sargent et al., 2000), although medication-naïve MDD patients have more 5-HT_{1A} binding in the midbrain and other brain regions (Parsey et al., 2006). Studies in non-human primates support the involvement of 5-HT_{1A} receptors in mood. In behaviorally depressed cynomolgus monkeys, 5-HT_{1A} BP measured with the 5-HT_{1A} antagonist, 4,2″-(methoxyphenyl)-1-[2″-(*N*-2″-pyridinyl)-*p*-fluorobenzamido] ethylpiperazine, was lower in raphe and other brain regions. (Shively et al., 2006). No in vivo studies have reported on suicidal behavior and 5-HT_{1A} binding.

The 5-HT synthesizing neurons in the brainstem dorsal (DRN) and median (MRN) raphe nuclei give rise to the serotonergic innervation of the forebrain. In the raphe, the 5-HT_{1A} receptor is a somatodendritic inhibitory autoreceptor on 5-HT neurons (Verge et al., 1985; Middlemiss and Fozard, 1983). Locally released 5-HT acts on the autoreceptor inhibiting neuronal firing (Wang and Aghajanian, 1977; De Montigny et al., 1984; Artigas et al., 1996; Hensler, 2003; Celada et al., 2001) and reducing 5-HT release in all projection targets (Aghajanian et al., 1987).

1.2. Objectives of the study

Previous studies yield conflicting results. Stockmeier et al. (1998) reported more 5-HT_{1A} binding in depressed suicides vs. controls in the ventrolateral and dorsal subnuclei of the DRN in rostral midbrain. When calculating binding density × total volume of the nucleus (binding capacity), we found lower 5-HT_{1A} receptor binding in depressed suicides (Arango et al., 2001). Differences in the study populations and different regions of the DRN being sampled between the two studies could account for the discrepant findings. We (Arango et al., 2001) included more female subjects and analyzed the caudal subnucleus, that was omitted by Stockmeier et al. (1998). Finally, we estimated the total amount of 5-HT_{1A} binding in the volume of the DRN and/or MRN using “binding capacity” as an index of the total number of receptors in the DRN or MRN where: binding capacity = (cross-sectional receptor binding in fmol/mg tissue) × (region volume). In suicides, the DRN binding capacity, but not cross-sectional receptor binding, was lower in suicides.

In postmortem (Arango et al., 1995) and in vivo PET studies, (Parsey et al., 2002, 2005) females have more 5-HT_{1A} receptor binding in PFC than males. No postmortem studies have considered sex in comparing 5-HT_{1A} binding in suicide and controls in the brainstem.

Given the conflicting results about 5-HT_{1A} receptor binding in MDD suicide victims, we sought to determine the possible effects of sex and of region of the DRN through an expanded regional analysis of 5-HT_{1A} receptor binding, assayed previously (Arango et al., 2001).

2. Methods

2.1. Subjects

The present study was carried out on subjects previously collected and reported on Arango et al. (2001) and their characteristics are only summarized here. The investigation was carried out in accordance with the latest version of the Declaration of Helsinki. Brain samples were obtained in accordance with Institutional Review Board requirements. Next-of-kin consented to tissue donation after full disclosure of the nature of the procedures.

All cases died suddenly. A review of available records and an interview with at least one informant per case was performed according to our psychological autopsy method (Kelly and Mann, 1996) to determine axis I and axis II diagnoses and other clinical information according to DSM-IV criteria.

Brains were collected at autopsy, dissected into blocks and flash-frozen in Freon (−20 °C) and stored at −80 °C until sectioning. Samples from the left cerebral hemisphere were fixed for neuropathologic examination. Individuals with a history of cerebral trauma, central nervous system disease, chronic alcoholism, or AIDS use were excluded. Body fluids (blood, bile, aqueous humor and urine) were used for toxicological screening for cocaine, opiates, alcohol, antidepressants and other acidic and basic drugs. The brain samples were coded and sections collected and assayed by personnel blind to the cause of death.

Suicides (n = 10) and controls (n = 10) were matched for age (±5 years), sex (same sex) and postmortem interval (PMI, ±5 h) and assayed as pairs. Controls did not meet criteria for axis I or axis II diagnosis during their lifetime (see Table 1). A history of prescribed medications during the last three months of life was also taken. Detailed demographic information on individuals is reported elsewhere (Arango et al., 2001).

2.2. Brainstem

Three sets of DRN sections every mm, previously assayed for 5-HT_{1A} receptors (2 slides; total and non-specific binding) by quantitative receptor autoradiography using ^{3}H-8-OH-DPAT (Fig. 1) and Nissl stain (thionin, 1 slide) were used. Each slide used for receptor autoradiography was also stained for Nissl substance.

Comparable rostrocaudal anatomical levels were identified. For each case, the tissue section with the maximum DRN binding area, measured in a ^{3}H-8-OH-DPAT autoradiogram, was identified (see Fig. 1, Section 2 in upper panel) and used to align the other sections every 1 mm (missing sections were considered as missing and the interval was calculated accordingly). For statistical analysis of the rostrocaudal distribution, the DRN was divided into 6 levels relative to the section with the greatest cross sectional area as described in a previous study (Boldrini et al., 2005): −3 mm (3 mm rostral from peak area), 0 mm (DRN peak area), 3 mm (3 mm caudal from peak area), 6 mm (6 mm caudal from peak area), 9 mm (9 mm caudal from peak area), 12 mm (12 mm caudal from peak area). The cross-sectional binding value for each level was derived from the average of three slides except for the peak level (0 mm) and the second minor peak level (6 mm), which contained two slides. Plotting DRN 5-HT_{1A} binding area on a graph with distance in mm from peak on the abscissa, we obtained a curve (Fig. 3A) which shows the same pattern of rostrocaudal variation of the DRN area obtained in previous studies performing TPH immunostaining (Underwood et al., 1999) and TPH immunoautoradiography (Boldrini et al., 2005). This was taken as a confirmation that the method of section alignment allowed for a representative across subject representation of DRN anatomical location.

2.3. Quantitative receptor autoradiography of [^{3}H]8-OH-DPAT to the 5-HT_{1A} autoreceptor

Autoradiography of 5-HT_{1A} receptors was performed as previously described, including exposure for two weeks and development (Arango et al., 1995, 2001). Tissue sections were fixed in 10% buffered formalin and stained for Nissl substance with thionin.

2.4. Densitometry of 5-HT_{1A} receptor autoradiograms

Receptor autoradiograms were quantified using a computer-based image analysis system (MCID, Imaging Research, Inc.), as previously described (Arango et al., 1995, 2001). Images of [^{3}H]8-OH-DPAT autoradiograms were used to outline the boundaries of the DRN since

there is a high degree of correspondence between the outline of the DRN based on sections immunostained for tryptophan hydroxylase (PH8, Törk and Hornung, 1990; Stockmeier et al., 1996) and the outline based on [^{3}H]8-OH-DPAT autoradiograms (Arango et al., 2001).

2.5. Binding capacity

We previously introduced the concept of binding capacity (Arango et al., 2001) as an index of the total binding in a brain area of interest. This was considered to be a more physiologically-relevant measure than the cross-sectional binding in a region of interest (e.g. fmol of radioactive ligand/mg of tissue) because it estimates the total amount of receptors and is also more comparable with data obtained *in vivo* by brain imaging. The total amount of binding is calculated as: binding capacity = (cross-sectional receptor binding) × (binding area × distance between sections) (fmol/mg tissue × mm^3).

2.6. Statistical analysis

Ten matched pairs of controls and suicide victims were used for the group by level analysis of [^{3}H]8-OH-DPAT binding density, binding area and binding capacity. ANOVA analysis for repeated measures was performed using, as the grouping variable, suicide/non-suicide and as a within-subjects factor, the six rostrocaudal levels measured. Univariate tests performed at each level were used to analyze group differences for [^{3}H]8-OH-DPAT binding density, binding area and binding capacity. The same analysis was also performed within male and female groups to analyze control-suicide differences in the two sexes.

Linear correlation analysis was used to analyze the relationship between film autoradiography measures and pH, age and PMI. Data are expressed as mean ± SD and all p values are 2-tailed.

3. Results

3.1. Cross-sectional binding

5-HT$_{1A}$ receptor cross-sectional binding (fmol/mg tissue) varies along the rostrocaudal axis of the DRN (F = 11.08 df = 5,14; p = 0.0002; Table 2; Fig. 2a). Binding is greatest rostrally from 0 to 6 mm and relatively less in caudal portions (Fig. 2a).

The suicide group shows a decline in cross-sectional binding proceeding from rostral to caudal compared with controls (Fig. 2a). The distribution of cross-sectional binding along the rostrocaudal axis of the DRN differs between depressed suicides and controls (Table 2). Univariate tests performed at each level show higher binding in depressed suicides at the most rostral −3 mm level (F = 39.63; df = 1,18; p = 0.0001), and lower binding at the 3 mm level (F = 5.75; df = 1,18; p = 0.0276) and the 6 mm level (F = 24.23; df = 1,18; p = 0.0001; Fig. 2a). At other levels, the differences are not statistically significant.

In females, a difference in cross-sectional binding was also found (F = 28.47; df = 5,2; p = .0343) and post hoc tests revealed higher [^{3}H]8-OH-DPAT binding in female depressed suicides compared to female controls at the most rostral DRN level (−3 mm, F = 19.60; df = 1,6; p = 0.0044), and lower binding at the more caudal 3 mm level (F = 17.94; df = 1,6; p = 0.0055) and 6 mm level (F = 9.00; df = 1,6; p = 0.0240; Fig. 2b). In males (Fig. 2c), cross-sectional binding differed between depressed suicides and controls (F = 8.59; df = 5,6; p = .0105) and upon post hoc testing, the depressed suicides had higher binding at the most rostral DRN level (−3 mm) (F = 23.69; df = 1,10; p = 0.0007) and lower at the more caudal end at the 6 mm level (F = 17.61; df = 1, 10; p = 0.0018; Fig. 2c).

When looking at sex differences within controls and within depressed suicides, we found that, in controls, there was a difference between males and females (F = 22.24; df = 5,4; p = .0051;

Fig. 2b and c). Control females have higher binding than control males, and the control male and female patterns differ ($F = 22.24$; df = 5,4; $p = .0050$; Fig. 2b and c). In depressed suicides, there was no sex difference in rostrocaudal distribution of cross-sectional binding ($F = 0.53$; df = 1,8; $p = .4892$) with binding decreasing caudally in both males and females. There was no sex by level interaction ($F = 4.33$; df = 5,4; $p = .0901$).

3.2. Binding area

A rostrocaudal variation in the area occupied by 5-HT_{1A} receptors exists in the DRN (Table 2; Fig. 3a). The binding area is greatest rostrally; there is a smaller peak at the 6 mm level. In depressed suicides there is less binding area from 0 to −3, and the second peak at 6 mm is almost missing compared with controls (Table 2; Fig. 3a). The total binding area is an approximation of DRN volume and is smaller in depressed suicides compared with controls (Table 2). Post hoc tests confirm smaller binding area in depressed suicides compared with controls at the most rostral DRN level (−3 mm) ($F = 26.79$; df = 1,18; $p < .0001$) and at the 6 mm level ($F = 7.19$; df = 1,18; $p = 0.0152$; Fig. 3a).

Analyzing sex differences in controls we found that [^{3}H]8-OH-DPAT binding area shows a comparable pattern of the area variation in the rostrocaudal axis ($F = 4.83$; df = 5,4; $p = 0.0762$), with no male to female differences in the total DRN area ($F = 0.14$; df = 1,8; $p = 0.7174$; Fig. 3b and c).

In depressed suicides however we found less [^{3}H]8-OH-DPAT binding area at all rostrocaudal levels in females compared to males ($F = 9.33$; df = 1,8; $p = 0.0157$) and the post-hoc test shows that the largest deficit in DRN area in female depressed suicides compared to male depressed suicides is at the level of greatest DRN area, rostrally at level 0 ($F = 5.33$; df = 1,8; $p = 0.0490$; Fig. 3b and c).

3.3. Binding capacity

A rostrocaudal variation in 5-HT_{1A} receptor binding capacity in the DRN is observed (Table 2; Fig. 4a) reflecting different areas and densities in the different DRN subregions. Binding capacity is lower in depressed suicides compared with controls (Table 2; Fig. 4a).

Among females, MDD suicides have lower [^{3}H]8-OH-DPAT binding capacity than controls ($F = 12.38$, df = 1,6; $p = .0125$) at the rostral DRN region (0 mm level) and at the level of enlargement of the caudal DRN subnucleus (6 mm level; Fig. 4b). In males, lower [^{3}H]8-OH-DPAT binding capacity was found in depressed suicides compared with controls ($F = 7.04$; df = 1, 10; $p = .0242$) at the most rostral −3 mm level and at the enlargement in the caudal portion of the DRN within the caudal subnucleus (6 mm level; Fig. 4c).

Binding capacity ($F = 0.06$, df = 1,8; $p = .8167$) was comparable in control males and females (Fig. 4b and c). In contrast, in depressed suicides females [^{3}H]8-OH-DPAT binding capacity was lower compared to suicide males ($F = 10.88$; df = 1, 8; $p = .0109$), showing a total flattening at the rostral DRN region (Fig. 4b and c).

3.4. Effect of clinical diagnosis, brain pH, postmortem interval (PMI) and age on 5-HT_{1A} receptor binding, binding area and binding capacity

No effects of brain pH (range 6.23–6.77; $p > 0.05$), PMI (time in hours from death to collection of brain tissue) or age were observed on 5-HT_{1A} receptor binding density, binding capacity or DRN binding area. Since all but one suicide had a diagnosis of MDD and was in a depressive episode around the time of death, we could not determine whether our findings are due to major depression or suicide.

4. Discussion

A difference in the anatomical distribution of 5-HT_{1A}receptor binding along the rostrocaudal axis of the DRN was observed in depressed suicides and non-suicide, control group, which is dependent on sex. The variation of 5-HT_{1A} receptor density along the rostrocaudal axis of the DRN was previously recognized (Stockmeier et al., 1996). We confirm and extend the observations of Stockmeier et al. (1996), examining the area of distribution of 5-HT_{1A} receptor binding (mm^2) and the binding capacity as the product of cross sectional area, distance between the sections of each level and binding density at that level [binding capacity = (cross-sectional receptor binding) × (region volume) = (fmol/mg tissue) × mm^3] together with the 5-HT_{1A} autoreceptor binding amount per unit tissue (fmol/mg tissue) along the full rostrocaudal extent of the DRN. This is a new observation since previous studies did not quantify area or volume in considering the total receptor binding, but only measured binding per mg protein (Stockmeier et al., 1998) or per mg tissue (Arango et al., 2001). 5-HT_{1A} receptor binding capacity varies along the rostrocaudal extent of the raphe and in depressed suicides is lower than in controls in both the rostral and the caudal DRN.

We found higher 5-HT_{1A} receptor cross-sectional binding (fmol/mg tissue) in the rostral DRN in depressed suicides compared to controls, consistently with the results obtained by Stockmeier et al. (1998) in the rostral raphe. 5-HT_{1A} receptor binding was lower in suicides compared to controls in the caudal subnucleus, thereby explaining why there was no between groups differences when averaging across the whole raphe values (Arango et al., 2001).

4.1. Effect of rostrocaudal DRN level

The rostralcaudal difference in cross-sectional binding could explain the apparent discrepancies in the literature (Stockmeier et al., 1998 versus Arango et al., 2001). Our previous study included the rostrocaudal extent of the raphe, from the superior colliculus to the middle cerebellar peduncle, but did not divide the DRN into anatomical regions as we have done in the present study. Consistent with Stockmeier et al. (1998) we find higher 5-HT_{1A} binding at the most rostral level of the DRN, which includes the interfascicular, ventral, ventrolateral and dorsal subnuclei (Boldrini et al., 2005) in depressed suicides compared with controls. In contrast, we also find 5-HT_{1A} binding is lower in depressed suicides at more caudal levels, where the caudal subnucleus of the DRN is located within the pontine portion of the raphe (Boldrini et al., 2005). The lower binding in depressed suicides relative to controls at more caudal levels may therefore offset the higher binding in depressed suicides at rostral levels, explaining why no difference was detected between depressed suicides and controls when the rostrocaudal level is not considered (Arango et al., 2001).

The observation of more or fewer 5-HT_{1A} receptors at discrete rostrocaudal levels of the DRN of depressed suicides compared to controls may be related to differences in the topographical pattern of efferent projections of the rostral versus caudal DRN. Most data about anatomical projections of the DRN come from rodent and monkey studies (Wilson and Molliver, 1991a,b). The elaboration of the DRN phylogenetically warrants caution in extrapolating these results to man. Nevertheless, in the rodent, neurons projecting to the caudate-putamen and substantia nigra are located in the rostral portion of the DRN, whereas neurons projecting to the hippocampus and locus coeruleus are more caudal (Imai et al., 1986). Neurons projecting to the medial prefrontal cortex in the rat are clustered in the medial DRN (Van Bockstaele et al., 1993). The anterior thalamic nucleus receives a serotonergic projection from the ventromedial and ventrolateral part of the ipsilateral DRN (Gonzalo-Ruiz et al., 1995), whereas the pyriform cortex is innervated by neurons in the ventromedial DRN (Datiche et al., 1995) The caudal DRN includes projections to the pineal gland in the golden hamster (Leander et al., 1998). In macaque monkeys, a coarse rostrocaudal topographic relationship is reported between dorsal raphe subnuclei and cortical targets. Projections to dorsolateral prefrontal

cortex are concentrated in the rostral part of the dorsal raphe nucleus (Wilson and Molliver, 1991b). Thus, overall, it is likely that rostral neurons in the DRN provide most projection to cortical regions. We hypothesize that the rostral elevation of DRN 5-HT_{1A} autoreceptor binding in depressed suicides is functionally related to our finding of more 5-HT_{1A} binding in the ventral and lateral prefrontal cortex (Arango et al., 1995) because an excess of autoreceptors would tend to lower firing rate and reduce serotonin release. Less serotonin release may result in potential upregulation of post-synaptic receptors in target regions such as prefrontal cortex.

The DRN 5-HT_{1A} receptor distribution corresponds closely with tryptophan hydroxylase protein immunoreactivity (Underwood et al., 1999) which also shows the larger cross-sectional area at the rostral end of the DRN complex. TPH2 mRNA (Bach-Mizrachi et al., 2006) and protein immunoreactivity are greater in the DRN of depressed suicides (Boldrini et al., 2005) although not all studies agree (Bonkale et al., 2004) and we (Underwood et al., 1999), but not others (Baumann et al., 2002) find more serotonin neurons in the DRN. The DRN 5-HT_{1A} receptor cross sectional area shows one large rostral peak and a second peak, approximately 6 mm caudal (caudal subnucleus). Depressed suicides in comparison to controls have a sharper drop off of 5-HT_{1A} receptor binding area starting from the most rostral portion of the DRN, and the second area enlargement is almost missing (Fig. 3A). The reason for fewer autoreceptors more caudally appears related to the presence of fewer DRN neurons expressing the 5-HT_{1A} receptor more caudally. What we cannot determine in this study is whether the binding level per neuron varies in the rostral-caudal axis or between depressed suicides and controls. A future study looking at binding levels per cell is needed to address this question.

Lower 5-HT_{1A} receptor expression, indicating less autoreceptor inhibition, despite more TPH biosynthetic enzyme and more TPH2 mRNA (Bach-Mizrachi et al., 2006), could suggest upregulatory effects at a neuron level with DRN neurons having more capacity for serotonin synthesis and less autoinhibition. Such an effect would enhance neuronal firing and may be a potential compensatory response for a lack of serotonin. The decrease in 5-HT_{1A} autoreceptor binding is also a similar effect as that of chronic SSRI administration to rodents (Blier and De Montigny, 1994). It has been hypothesized that this autoreceptor downregulation or desensitization effect is the basis for the therapeutic action of SSRIs (Blier and De Montigny, 1994). Perhaps the decrease in autoreceptor binding we observe in depressed suicides is a homeostatic effect functionally similar to that produced by SSRIs, although not sufficient, to restore serotonin levels.

4.2. Binding capacity

Cross-sectional binding and area vary along the rostrocaudal axis of the DRN. The rostral portion of the DRN contains the dorsal, ventral, ventrolateral and Interfascicular subnuclei at levels −3 mm to 3 mm, while the caudal DRN contains the caudal and interfascicular subnuclei (levels 3–12 mm). We calculated binding capacity as an index of the total amount of binding in the rostral and caudal DRN to account for variations between subnuclei.

In depressed suicides, binding capacity in the DRN is lower than in controls in both the rostral and the caudal DRN, This is consistent with the in vivo PET finding that 5-HT_{1A} receptor binding potential was 42% lower in the raphe nuclei of depressed subjects compared to controls (Drevets et al., 1999). This PET study used the antagonist 11-C-WAY100635, while we used the agonist 8-OH-DPAT in our *in vitro* studies. If we assume that both the PET and our postmortem studies are correct, depressed suicides have both lower high affinity agonist and low affinity antagonist binding. Since the high affinity conformation of the receptor is coupled to the G protein, agonist binding is a measure of the signal transduction capacity and lower agonist binding is also consistent with lower sensitivity of the autoreceptor. Lower 5-HT_{1A} receptor binding potential was also associated with life-time aggression (Parsey et al., 2002), and since greater aggression is associated with suicidal behavior, that finding is also consistent

with our postmortem findings, but suggests the relationship is with suicidal behavior rather than with major depression. That conclusion is also supported by our PET studies finding higher binding in medication-naïve major depression patients (Parsey et al., 2006). This may suggest that suicide or aggression may explain the low postmortem 5-HT_{1A} binding observed in our sample. Future studies on suicides with a different psychiatric diagnosis and depressed subjects not dying by suicide are necessary to test this hypothesis.

4.3. Effect of sex

We found a sex effect on 5-HT_{1A} binding that was independent of the difference between groups. Within controls, females have more 5-HT_{1A} binding than males. Within suicides, 5-HT_{1A} binding is lower in females than in males.

No previous postmortem study has analyzed suicide/control differences on 5-HT_{1A} receptors binding between sexes in the brainstem. In cerebral cortex, we previously reported higher prefrontal [^{3}H]8-OH-DPAT binding in females compared to males (Arango et al., 1995) with the difference being up to 40% in some prefrontal cortical sub regions. A PET study showed that healthy females have higher 5-HT_{1A} [^{11}C]WAY-100635 BP than males in the cortex and DRN (Parsey et al., 2002) and in the cerebellum (Parsey et al., 2005) a finding consistent with our results here. Since estradiol increases 5-HT_{1A} binding (Biegon et al., 1982; Biegon and McEwen, 1982), such an effect may explain the sex differences of higher 5-HT_{1A} receptor binding seen in females in postmortem and PET imaging studies (Parsey et al., 2002, 2005).

It appears that these binding differences have functional consequences. Ipsapirone, a 5-HT_{1A} partial agonist, stimulates GH secretion more in male than in female subjects indicating greater responsivity in females (Newman et al., 1999). Animal studies suggest different 5-HT_{1A} responses in males and females (Gelfin et al., 1995; Ebenezer and Tite, 1994; Haleem et al., 1989, 1990; Joppa et al., 1997; Uphouse et al., 1991; Mendelson and McEwen, 1991; Curzon, 1989). 5-HT transporter knockout mice have lower 5-HT_{1A} receptor binding in the dorsal raphe and this is more extensive in females compared with males (Li et al., 2000) suggesting that females may have a stronger compensatory response to higher intra-synaptic serotonin levels.

Due to the limited number of subjects studied, conclusion regarding the male/female differences should be considered as tentative and the findings need to be replicated in larger samples.

4.4. Limitations

Confounding factors potentially influencing 5-HT_{1A} binding results include age and PMI. However, we matched control and suicide subjects for these variables and did not find significant correlations of either of these parameters with binding measures. Another confounding factor may be the use of drugs or medications that may desensitize 5-HT_{1A} receptors or alter 5-HT_{1A} receptor density and their G-protein coupling (Le Poul et al., 1995; Blier and De Montigny, 1994; Davidson and Stamford, 1998; Stockmeier et al., 1998; Rossi et al., 2006). In the present study, no subjects were taking antidepressants at the time of death, therefore the observed differences are not likely attributable to medication. We found higher binding in the rostral DRN, which is the opposite direction of change that would be predicted from animal studies of the effects of SSRIs on 5-HT_{1A} autoreceptors (Riad et al., 2004; Aznavour et al., 2006). Also, the direction of change was different in the caudal DRN where depressed suicides had less binding, a complex pattern of abnormality making a medication effect an unlikely explanation.

Since 90% of the suicides in our sample also had a history of major depression, our findings may be due to major depression or the diathesis for suicide. Most postmortem brainstem studies of suicide analyzed subjects that, in life, had experienced an episode of major depression (Arango et al., 2001; Underwood et al., 1999; Stockmeier et al., 1998) but the serotonin system abnormalities associated with mood disorders and with suicide may be different as seen for example in the prefrontal cortex. A diffuse decrease in serotonin transporter binding throughout the dorsoventral extent of the prefrontal cortex was found in association with major depression, while a localized reduction in ventral prefrontal cortex transporter binding was associated with suicide (Mann et al., 2000). Future studies must determine whether serotonergic alterations observed at the level of the DRN are specific for suicide or related to mood disorders and whether different parts of the DRN or components of the serotonin system are related to depression or suicide.

A major limitation of the study is the small sample size. Given the complexity of 5-HT_{1A} receptor changes due to anatomical location and sex, future studies should seek to replicate the present findings.

5. Conclusions

The current study demonstrated anatomically distinct changes in DRN 5-HT_{1A} receptor binding of MDD suicides compared with controls. 5-HT_{1A} receptor binding is higher in MDD suicides in the rostral DRN and lower in the caudal DRN compared to controls. This indicates that 5-HT_{1A} receptor expression is heterogeneously affected in MDD and/or suicide, and that the level of autoreceptor expression within functionally diverse DRN subregions may have consequences for projections to specific brain regions. 5-HT_{1A} receptor binding is higher in females than males suggesting greater autoreceptor inhibition in females, reduced serotonergic neurotransmission and the potential for greater risk for effects on mood related to 5-HT function. MDD suicide females have fewer 5-HT_{1A} receptors than MDD suicide males and this may suggest greater homeostatic responsivity in females than males.

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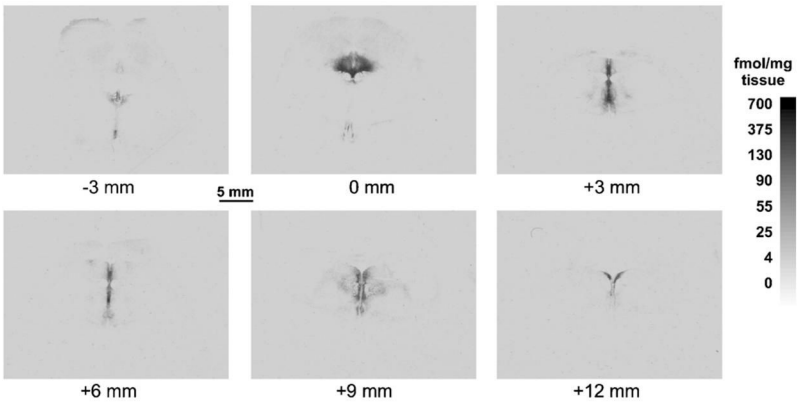

Fig. 1.

A series of immunoautoradiograms illustrating the rostrocaudal distribution of 5-HT_{1A} receptor binding in a representative brain. The largest part of the dorsal raphe nucleus is indicated as the origin (0 mm) and the other five levels are measured as actual distance (in mm) rostral and caudal to the origin.

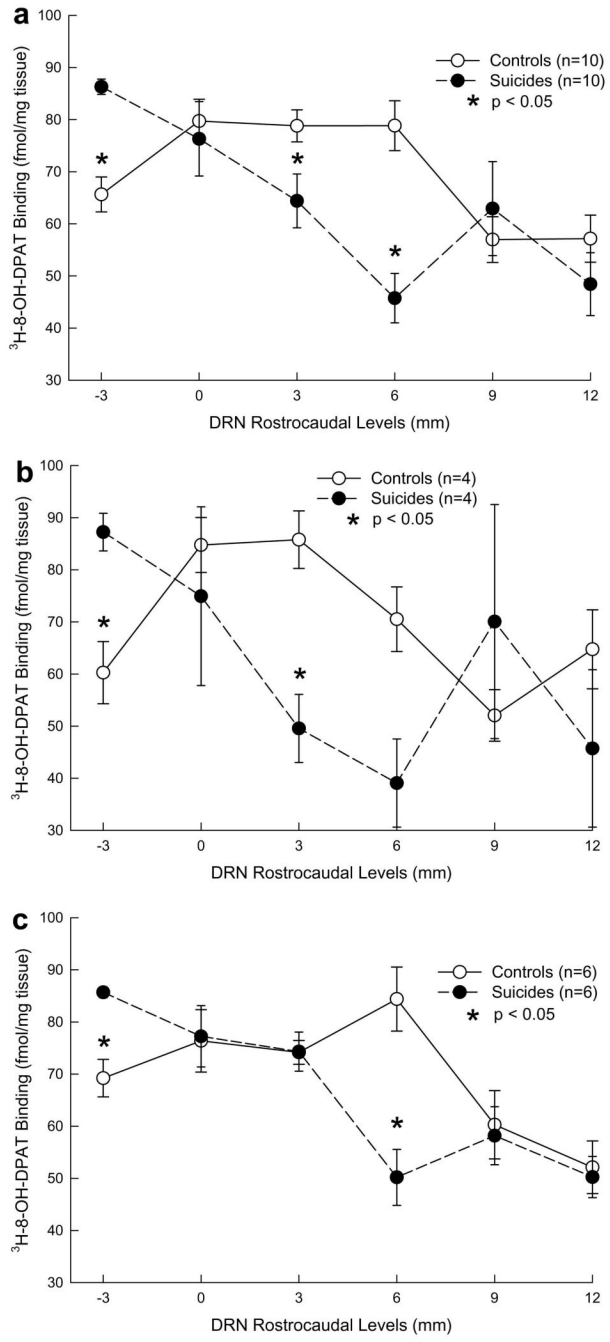

Fig. 2.

5-HT$_{1A}$ receptor ([^{3}H]-8-OH DPAT) binding density in controls and depressed suicides in total sample (a), in females (b) and in males (c) at each dorsal raphe nucleus rostrocaudal level. Binding density is higher in the rostral raphe and lower in the caudal raphe in depressed suicides compared to controls. In controls, 5-HT$_{1A}$ receptor binding is higher in females than in males. In suicides, 5-HT$_{1A}$ binding is lower in females than in males.

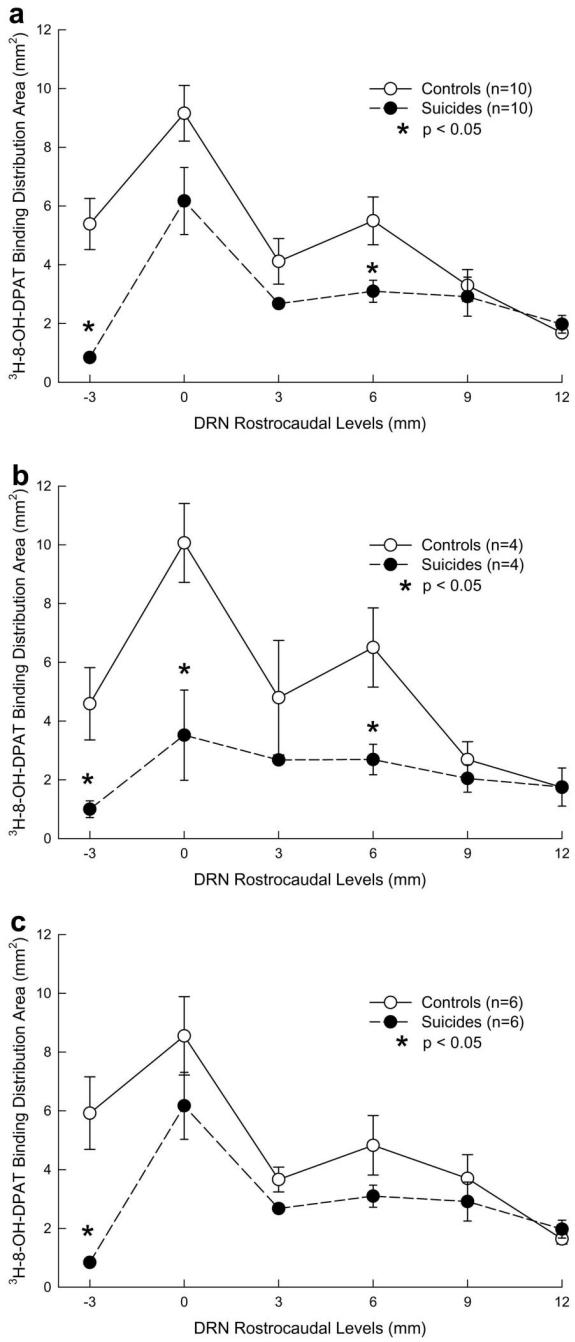

Fig. 3.

5-HT$_{1A}$ receptor ([^{3}H]-8-OH0DPAT) binding distribution area in controls and depressed suicides in total sample (a), in females (b) and in males (c) at each dorsal raphe nucleus rostrocaudal level. Binding distribution area is lower in depressed suicides than in controls. In females, Area is lower in depressed suicides at most rostrocaudal levels compared to controls. In males, Area is lower only at the most rostral level in suicides versus controls. Distribution area is not affected by sex in controls. Female suicides have lower distribution area than male suicides.

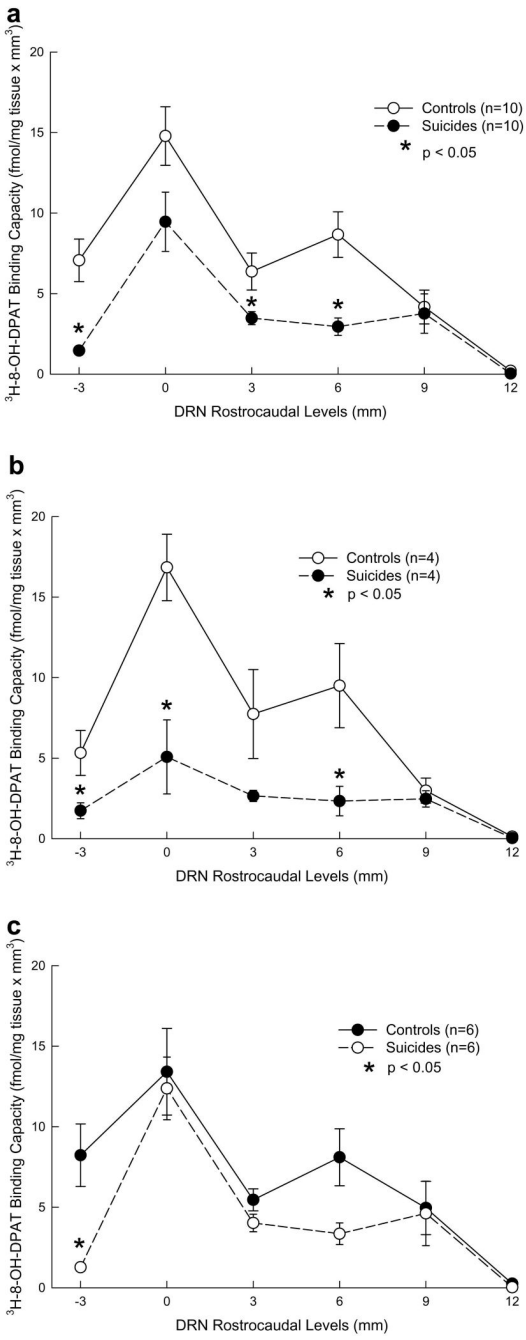

Fig. 4.

5-HT$_{1A}$ receptor [^{3}H]-8-OH DPAT binding capacity in controls and depressed suicides in total sample (a), in females (b) and in males (c) at each dorsal raphe nucleus rostrocaudal level. Binding capacity is lower in depressed suicides than in controls, and this is true at all levels in females, and more selectively in males. In controls, binding capacity is higher in females than in males, but in suicides, binding capacity is lower in females than in males.

Table 1

Demographics, diagnosis and toxicological screening results of suicides and controls

	Controls (*n* = 10)	**Suicides (*N* = 10)**
Sex (M:F)	6:4	6:4
Age (years)	45.4 ± 18.6	44.1 ± 7.7
PMI (h)	12.7 ± 4.9	15.8 ± 2.2
Brain pH	6.4 ± 0.3	6.4 ± 0.3
Race (C:H:AA:As)	7:1:2:0	6:3:0:1
Toxicology	10 = nothing detected 1 = carbon monoxide	8 = nothing detected 1 = opiates 1 = analgesic 1 = opiates, analgesics
Alcoholism	0	0
Axis I Diagnosis	None	7 = MDD 1 = Schizoaffective, MDD 1 = MDD, Bulimia
Axis II Diagnosis	None	1 = Schizotypical Personality Disorder
Cause of death	5 = cardiovascular 4 = motor vehicle accident (pedestrian) 1 = motor vehicle accident (passenger)	5 = gun shot wound 2 = hanging 3 = fall from height

PMI, postmortem interval; C, caucasian; H, hispanic; AA, African American; As, Asian; MDD, Major Depressive Disorder.

Table 2

5-HT_{1A} binding density, binding distribution area and binding capacity changes in the dorsal raphe nucleus (DRN) depending on group membership (depressed suicides *versus* controls) and DRN rostrocaudal level

Measure	Factors	*df*	*F*	p
5-HT_{1A} binding (fmol/mg tissue)	Group	1,18	2.20	.1555
	Level	5,14	11.08	.0002
	Group * Level	5,14	11.61	.0001
5-HT_{1A} binding distribution area (mm^2)	Group	1,18	14.76	.0012
	Level	5,15	14.85	<.0001
	Group * Level	5,14	4.19	.0154
5-HT_{1A} binding capacity (fmol/ mg tissue* mm^3)	Level	5,14	26.62	<.0001
	Group	1,18	18.06	.0005
	Group * Level	5,14	5.05	.0075

Group = depressed suicides vs. controls; Level = DRN rostrocaudal level of tissue sections; Group * Level = group by rostrocaudal level interaction.