BRIEF REPORT

Decreased Neurokinin-1 (Substance P) Receptor Binding in Patients with Panic Disorder: Positron Emission Tomographic Study with [^{18}F]SPA-RQ

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Background: Positron emission tomography (PET) can localize and quantify neurokinin-1 (NK_1) receptors in brain using the nonpeptide antagonist radioligand, [^{18}F]SPA-RQ. We sought to determine if patients with panic disorder have altered density of NK_1 receptors in brain because of their history of recurrent panic attacks. We also sought to determine if a drug-induced panic attack releases substance P in brain, as measured by decreased binding of [^{18}F]SPA-RQ.

Methods: Positron emission tomography scans with [^{18}F]SPA-RQ were performed in 14 patients with panic disorder and 14 healthy subjects. Of these two groups, 7 patients and 10 healthy subjects were scanned twice, once at baseline and once after injection of doxapram, a drug that induces panic attacks.

Results: NK_1 receptor binding in patients ($n = 14$) compared with that in healthy subjects ($n = 14$) was significantly decreased by 12% to 21% in all brain regions. Doxapram effectively produced panic attacks in 6 of 7 patients with panic disorder but only 2 of 10 healthy subjects. Doxapram caused no significant change of [^{18}F]SPA-RQ binding in either patients or healthy subjects.

Conclusions: Although induction of a panic attack has no significant effect on [^{18}F]SPA-RQ binding to NK_1 receptors, patients with panic disorder have widespread reduction of NK_1 receptor binding in brain.

Key Words: NK_1 receptor, panic attack, panic disorder, positron emission tomography (PET), substance P

Because numerous animal studies have shown that pain and stress induce release of substance P in brain (1), we sought to determine whether neurokinin-1 (NK_1) (substance P-preferring) receptors are altered in the brains of patients with panic disorder (i.e., in patients with a history of recurrent and severe stress). We also sought to extend the provocative finding that acute stress in humans causes the release of substance P, as indirectly measured by decreased radioligand binding to NK_1 receptors. Michelgard *et al.* (2) found that viewing phobic pictures in patients with specific phobias was associated with decreased binding in right, but not left, amygdala. These intriguing results were interpreted as stress-releasing substance P, which then displaced radioligand binding.

SPA-RQ is a nonpeptide antagonist selective for human NK_1 receptors and is competitively displaced in vitro by substance P (3). We performed positron emission tomography (PET) scans with [^{18}F]SPA-RQ for the two aims of this study. First, we compared baseline scans of patients with panic disorder with healthy control subjects to determine whether this recurrent anxiety disorder is associated with altered density of NK_1 receptors. Repeated release of substance P could, for example, down-regulate the number of NK_1 receptors. Second, to assess whether stress releases substance P in human brain, we imaged patients with panic disorder and healthy subjects twice: at baseline and after injection of doxapram, a respiratory stimulant known to induce panic attacks (4).

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Methods and Materials

Subjects

We studied 14 healthy subjects (36 ± 14 years; 9 female subjects and 5 male subjects) and 14 patients with panic disorder (35 ± 9 years; 9 female patients and 5 male patients). Values are mean ± SD. All healthy subjects lacked medical or psychiatric illness, based on history, physical examination, laboratory tests, and Structured Clinical Interview for DSM-IV-Nonpatient (SCID-NP).

Based on Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) interview and review by a psychiatrist (D.S.P.), all 14 patients met DSM-IV criteria for panic disorder. The average duration of panic disorder was 10 ± 8 years. The mean total score of the Panic Disorder Severity Scale (5) was 8.1 ± 2.9. Of the 14 patients, 13 patients had mild (less than one full panic attack per week) and one patient had moderate (one or two full panic attacks per week) severity of illness. Two patients were currently comorbid for agoraphobia and/or social phobia. Nine of 14 patients were drug naïve. Prior to the PET scan, all patients had not taken psychotropic medications (e.g., lorazepam) for a minimum of 3 weeks.

PET Imaging and Analysis

[^{18}F]SPA-RQ, produced as previously described (6), was obtained in high radiochemical purity (>99%) and specific activity at time of injection was 53 ± 20 GBq/μmol. After a transmission scan, [^{18}F]SPA-RQ (319 ± 58 MBq) was injected intravenously over ~1 min, and subjects were scanned on a GE Advance

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camera (GE Healthcare, Waukesha, Wisconsin). Brain radioactivity was measured in three intervals: 0 to 120 min, 160 to 180 min, and 220 to 240 min.

To identify regions in brain, we applied a standard template to the co-registered PET and magnetic resonance imaging (MRI) (T1-weighted) of each subject. Radioactivity in the reference (cerebellum) and eight target regions of brain (frontal cortex, lateral temporal cortex, parietal cortex, occipital cortex, cingulate, medial temporal cortex, striatum, and thalamus) were obtained with a template-based method, as described previously (7).

We quantified brain uptake as binding potential (BP_{ND}), which equals the ratio at equilibrium of specific to nondisplaceable brain uptake (8). The cerebellum was used as the reference region to measure nondisplaceable uptake. We used a two-parameter multilinear reference tissue model (9), which is relatively resistant to noise and has good reproducibility (~4% to 6%) in test studies of [^{18}F]SPA-RQ in healthy human subjects (10).

Doxapram Challenge

While we initially planned that all subjects would have two scans, we reviewed our data after studying 17 subjects, 8 of whom experienced panic attacks. This initial review suggested that the panic attack had insignificant effects on NK_1 receptor binding. To minimize exposing subjects to panic attacks, we terminated this phase of the experiment but finished studying a total of 14 healthy subjects and 14 patients at baseline.

Placebo (normal saline) was injected before the first PET scan, and doxapram (.5 mg/kg intravenous [IV] in 10 mL saline over 15 sec) was injected before the second PET scan, with both administered 30 min prior to [^{18}F]SPA-RQ. The duration between the first and second scans was 30 ± 38 days.

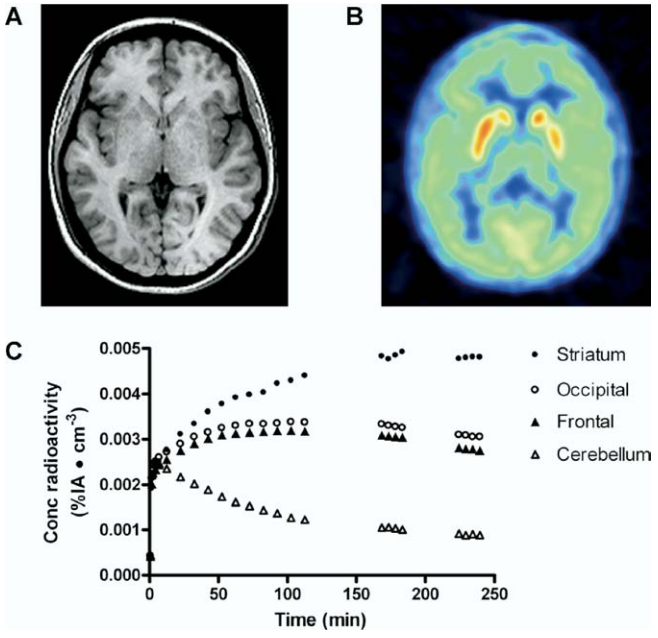

Figure 1. Distribution and time course of radioactivity in brain of a healthy subject after injection of [^{18}F]SPA-RQ. **(A)** The horizontal PET image is the sum of radioactivity from 60 min to 240 min after injection and shows high uptake in caudate and putamen. **(B)** This representative MRI image shows the anatomic landmarks corresponding to the PET image. **(C)** The concentration of radioactivity in striatum (●), occipital cortex (○), frontal cortex (▲), and cerebellum (△) are shown for the 240 min after injection of [^{18}F]SPA-RQ. MRI, magnetic resonance imaging; PET, positron emission tomography.

Table 1. Widespread Decrease of NK_1 Receptor Binding (BP_{ND}) in Patients with Panic Disorder Compared with Healthy Control Subjects

Region	BP_{ND} (mean ± SD) Control (n = 14)	Patient (n = 14)	% Decrease	p Value
Frontal	1.88 ± .30	1.53 ± .19	19	.0011
Lateral Temporal	2.03 ± .30	1.72 ± .19	15	.0032
Parietal	2.16 ± .33	1.76 ± .21	19	.0007
Occipital	2.14 ± .31	1.72 ± .20	20	.0002
Cingulate	1.85 ± .30	1.53 ± .20	17	.0026
Medial Temporal	1.47 ± .19	1.19 ± .20	19	.0008
Striatum	4.65 ± .73	4.10 ± .67	12	.0478
Thalamus	1.32 ± .25	1.04 ± .32	21	.0182

BP_{ND}, binding potential; NK_1, neurokinin-1.

We monitored symptoms of patients and healthy subjects during both PET scans using the Panic Symptom Scale (11) and Beck Anxiety Inventory score (12) at the following time points: ~30 min before the infusion of doxapram or saline and at 2 min, 10 min, and 20 min after infusion.

Results

After injection of [^{18}F]SPA-RQ, the regional distribution of radioactivity reflected the known distribution of NK_1 receptors (13): highest in striatum, intermediate in neocortex, and low in cerebellum (Figure 1).

NK_1 receptor binding at baseline in patients with panic showed widespread, statistically significant decreases compared with that in healthy control subjects (Table 1 and Figure 2). All eight receptor-rich brain regions had decreases of 12% to 21%, each with a p value $< .05$. Since a total of eight regions were examined, we applied a Bonferroni correction of 8, and the threshold was $p < .05/8 = .006$. Even with this stringent correction for multiple comparisons, the decreased binding in panic disorder remained significant in all regions except striatum and thalamus. We sought to determine if decreased binding in patients correlated with symptom severity but wished to avoid false-positive results that might occur by using all eight brain regions. For this reason and since all regions showed decreased binding, we created a single measure of receptor binding that was a volume-weighted average of all eight regions in 14 patients. In fact, radioligand binding in total brain was not correlated with years of illness, frequency of panic attacks, or total score on the Panic Disorder Severity Scale. Since BP_{ND} is the ratio at equilibrium of specific to nondisplaceable uptake (i.e., cerebellum), we compared uptake in cerebellum in patients and healthy subjects. The mean time activity curves in cerebellum of

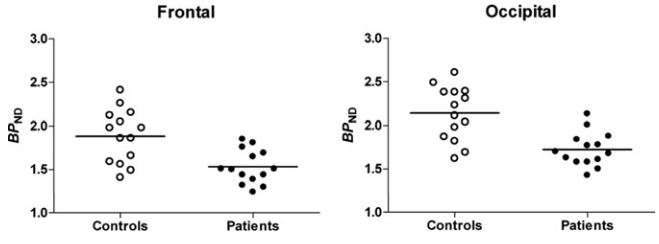

Figure 2. Decreased NK_1 receptor binding at baseline in patients compared with healthy control subjects. The distribution of receptor binding (BP_{ND}) in 14 patients and 14 healthy control subjects is shown for two brain regions (frontal and occipital). BP_{ND}, distribution of receptor binding; NK_1, neurokinin-1.

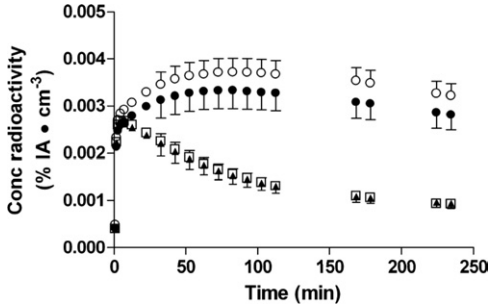

Figure 3. Uptake of radioactivity in cerebellum and occipital cortex of patients and control subjects at baseline. The magnitude and time course of radioactivity in cerebellum was virtually identical in patients with panic disorder (▲) and in healthy subjects (□). In contrast, uptake in occipital cortex of patients (●) was significantly less than that in healthy subjects (○). The density of target sites was quantified as BP_{ND}, which estimates the ratio of the area under the curve from time 0 to infinity in target region (which contains receptors) to that in a reference region (which has negligible receptors). $BP_{ND} = (AUC_{target}) / AUC_{reference}) - 1$. For occipital cortex, BP_{ND} in patients was 20% less than that in healthy subjects ($p = .0002$; Table 1). Symbols display mean values in 14 patients and 14 healthy subjects. To decrease clutter, the graph displays SEM rather SD, and some bars were omitted. The SEM for healthy subjects is shown above their symbols, whereas the SEM for patients is shown below their symbols. The concentration of radioactivity is expressed as % injected activity (IA) per cm^3 of tissue. AUC, area under the curve; BP_{ND}, distribution of receptor binding; IA, injected activity; SD, standard deviation; SEM, standard error of the mean.

these two groups were virtually identical (Figure 3). Doxapram effectively produced a panic attack in 6 of 7 patients with panic disorder but in only 2 of 10 healthy subjects. Symptoms of the panic attack typically began 1 to 2 min after injection, reached maximal magnitude within 10 min, and largely resolved by 20 to 30 min (Figure 4). The panic attack in 2 of the 10 healthy subjects had similar symptoms and duration but about half the magnitude of that in patients.

Doxapram had insignificant effects on radioligand binding in all the regions in the six patients who had a panic attack (Table 2) and in the entire group of 7 patients and 10 healthy subjects that received doxapram. Similar to other brain regions in our study, binding in right and left amygdala were insignificantly affected by panic attack (Table 2).

Table 2. Panic Attack Induced No Significant Change of NK_1 Receptor Binding (BP_{ND}) in Patients for whom Doxapram Induced a Panic Attack

Region	BP_{ND} (mean ± SD)		% Change
	Saline	Doxapram	
Frontal	1.60 ± .19	1.66 ± .16	3.6
Temporal	1.74 ± .16	1.81 ± .18	3.8
Parietal	1.85 ± .20	1.97 ± .21	6.2
Occipital	1.77 ± .23	1.86 ± .17	5.1
Cingulate	1.60 ± .22	1.62 ± .20	1.3
Medial Temporal	1.26 ± .21	1.29 ± .18	2.5
Striatum	4.38 ± .65	4.26 ± .61	−2.9
Thalamus	1.06 ± .20	1.10 ± .20	3.9
Right Amygdala	1.96 ± .25	2.03 ± .30	4.0
Left Amygdala	1.99 ± .44	1.86 ± .34	−6.3

BP_{ND}, binding potential; NK_1, neurokinin-1.

Discussion

We found that NK_1 receptor binding at baseline in patients with panic disorder was decreased in all brain regions by 12% to 21% compared with that in healthy control subjects. In addition, we found that doxapram-induced panic attack had insignificant effects on [^{18}F]SPA-RQ binding in brain.

Initial clinical studies suggested that NK_1 antagonists may have anxiolytic effects, but subsequent larger trials found no such effect. For example, one small trial found that an NK_1 antagonist was as useful to treat social phobia (14), and the initial placebo-controlled study of the NK_1 antagonist, aprepitant, showed both antidepressant and anxiolytic effects in patients with depression (15). Nevertheless, subsequent large multicenter studies showed that aprepitant lacked efficacy as an antidepressant and that the anxiety-based items in the Hamilton depression scales did not show an anxiolytic effect (16).

Our doxapram challenge study sought to extend the provocative finding that phobic stimuli decrease radioligand binding to NK_1 receptors in amygdala (2). Pain and stress in rodents release substance P in spinal cord and brain, which then causes internalization of postsynaptic NK_1 receptors, which can be blocked with NK_1 receptor antagonists (17,18). In patients with posttraumatic stress disorder (PTSD) and major depression, the concentration of substance P in cerebrospinal fluid is elevated by stressful stimuli (19).

In our provocation study, we found that doxapram effectively produced panic attacks in the majority of patients. Nevertheless, [^{18}F]SPA-RQ binding in brain was unchanged by doxapram. Thus, our results are inconsistent with the report of Michelgard *et al.* (2). We do not know the reasons for our negative findings, which might have been caused merely by our small sample size (8 patients with panic attack). However, their results may not be that robust, since they reported only two regions of brain, and results in right, but not left, amygdala were significant at $p < .05$. These results would presumably not withstand correction for multiple comparisons. Nevertheless, our negative results could also reflect the rapidity of substance P release and the slow kinetics of [^{18}F]SPA-RQ.

Conclusion

Although we found no acute effect of drug-induced panic attack on radioligand binding to NK_1 receptors, patients with panic disorder have widespread loss (12% to 21%) of radioligand binding to NK_1 receptors in brain.

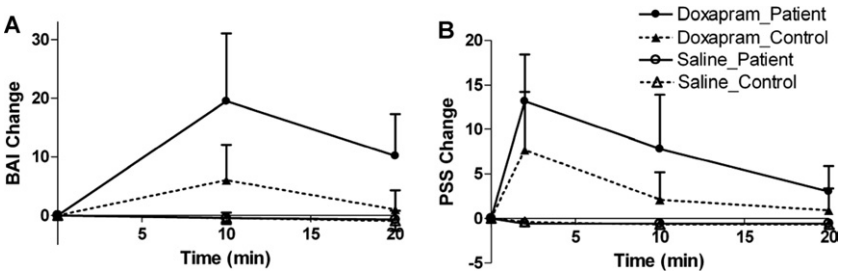

Figure 4. Change of anxiety and panic attack symptoms caused by doxapram or saline. **(A)** change of Beck Anxiety Inventory (BAI) score; **(B)** change of Panic Symptom Scale (PSS) score.

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