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Complicated Case Study

Diminished $GABA_A$ Receptor-Binding Capacity and a DNA Base Substitution in a Patient with Treatment-Resistant Depression and Anxiety

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In this report, we describe the case of a caucasion male patient, aged 42 years, suffering from severe treatment-resistant generalized anxiety disorder with panic attacks and from major depression for which he was treated with a course of electroconvulsive therapy. During electroconvulsive treatment, anesthesia was difficult to induce with etomidate and, once, propofol. Bispectral indices recordings (assessing the depth of anesthesia) revealed a much shorter duration of loss of responsiveness compared to a control patient receiving also a course of electroconvulsive therapy. Since $GABA_A$ receptors with ^{123}I-iomazenil SPECT and found a clearly diminished binding of the radiotracer in the right frontal and orbitotemporal regions compared to the recordings in a 38-year-old healthy male control. Genetic analysis of the exons 7 and 8 of the GABRB1–3 genes coding for the1–3-subunits of the $GABA_A$ receptors revealed a silent G to A substitution in the third position of amino acid 257 of the b1-subunit. To our knowledge, this is the first report of a link between insensivity to anesthetic agents and altered $GABA_A$ receptor function in a clinical case. Whereas reduced $GABA_A$ receptor-binding capacity has been investigated in anxiety disorders, this has not been the case in depressive disorders. This case illustrates how clinical observations in psychiatry can prompt investigations by modern techniques and potentially link clinics and basic sciences. No conclusion can, however, be made about casual links in this single case.
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INTRODUCTION

$GABA_A$ receptors are the target molecules of benzodiazepines and are part of the most important fast inhibitory neurotransmitter system in the brain. They are made up of five subunits (Olson, 2002). Whereas the most important and most prevalent $GABA_A$ receptor in the brain is made up from α1, β2, and γ2 subunits, at least 19 different subunits have been identified in mammals (Nutt and Malizia, 2001). Dysfunctions of these receptors have been implicated in different neurological and psychiatric disorders, especially anxiety disorders (Nutt and Malizia, 2001). Contrary to the body of evidence gathered about the binding capacity of $GABA_A$ receptors in different anxiety disorders by functional imaging techniques like ^{11}C-flumazenil PET and ^{123}I-iomazenil SPECT, this has not yet been studied in depression (Sanacora *et al*, 2000). In a recent report, however, the influence of electroconvulsive therapy (ECT) on the $GABA_A$ receptor-binding capacity in five patients suffering from major depression was studied using ^{123}I-iomazenil SPECT imaging. The binding capacity significantly increased 7 days after a mean of seven ECT sessions compared to a baseline value registered before the ECT course bilaterally in frontal, parietal, and occipital regions as well as in the right prefrontal area (Mervaala *et al*, 2001).

$GABA_A$ receptor-mediated regulation of cortical excitability is important with respect to epilepsy and general anesthesia. Although the precise mechanisms of central anesthetic agents are largely unknown, a recent study indicates that their sedative component might be mediated by $GABA_A$ receptors in rats (Nelson *et al*, 2002). Using gene-targeting strategies, changes of single amino acids in subunits of the $GABA_A$ receptor have been demonstrated to attenuate the action of general anesthetics *in vitro* and *in vivo* (Jurd *et al*, 2003; Nishikawa *et al*, 2002). Propofol potentiation of GABA-induced currents was abolished by a point mutation in the $GABA_A$ receptor β1 subunit (Krasowski *et al*, 1998). Etomidate has also been shown to influence the function of recombinant human $GABA_A$ receptors *in vitro* (Belelli *et al*, 1997).

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CASE REPORT

This Caucasian male patient, aged 42 years, weighing 78 kg, had a 6-year history of severe treatment-resistant generalized anxiety disorder with repeated panic attacks. A comorbid episode of severe major depression with somatic symptoms lasted 2 years, during which the patient attempted to commit suicide twice (first: cutting the left wrist; second: jumping from a bridge of a height of 8 m on a highway). During this time the patient met criteria for alcohol and benzodiazepine abuse. His father had died from suicide, two sisters suffered from major depression, and a brother from anxiety disorder. A grandmother also suffered from major depression. The patient was admitted to our Electroconvulsive Treatment unit. At admission, the rating of major depression as assessed with the Hamilton 21-item scale was 33 and Beck depression inventory was 37. Before the start of a course of ECT, he was treated with 263 mg clomipramine/day, 75 mg amitriptylin/day, 36.6 mmol lithium/day, and 1 mg alprazolam/day. While all other drugs were stopped prior to the course of electroconvulsive treatment, a medication with 225 mg clomipramine was maintained.

Generally, 0.5–1 mg alfentanil was given intravenously prior to induction of anesthesia at all of the treatment sessions. The induction of anesthesia with etomidate appeared to be extremely difficult in this patient during the first nine treatment sessions. A persistent deviation of the eye axis was interpreted as a sign of inadequate depth of anesthesia, and substantial myocloni made the assessment of depth of anesthesia difficult. This resulted in the administration of high doses of etomidate (first session of electroconvulsive treatment: 29 mg of etomidate; second session: 40 mg; third session: 60 mg; fourth session: induction with 450 mg propofol, no etomidate was administered; fifth session: 80 mg of etomidate; sixth session: 70 mg; seventh session: 78 mg; eighth session: 60 mg; ninth session: 80 mg). Muscle relaxation was always achieved with a dose of 50 mg succinylcholine. For the remaining seven sessions, a bispectral index monitor (Aspect A1000 monitor Bispectral Index™ version 3.2, Aspect Medical Systems Inc., Nattick, MA, USA) was used to assess the depth of anesthesia and it was discovered that the patient responded in a very unusual way to the anesthetic medication: while loss of responsiveness was achieved rather quickly, substantial myocloni obscured the clinical picture and the patient returned extremely quickly to consciousness again (Figure 1). In previous treatments this was interpreted as inadequate sedation and large additional doses of the anesthetic were administered. In the subsequent sessions, anesthesia could therefore be induced with standard doses of 0.26–0.38 mg etomidate/kg body weight only. Bispectral indices recorded in both the patient and a 41-year-old female control patient who also underwent ECT treatment are shown in Figure 1. In the index patient, loss of responsiveness, loss of eyelash reflex, and return of responsiveness occurred 25, 42 s, and 5 min 18 s after the administration of etomidate, respectively, whereas the same observations were made in the control after 31, 67 s, and 15 min 15 s. The maximal depth of anesthesia was 34 in the index patient and 28 in the control.

At 5 weeks after the last ECT session, a ^{123}I-iomazenil SPECT was performed in this patient and a 38-year-old healthy male control (Figure 2). Medication in our patient was stable for this time. At 10 min before an injection of 150 MBq ^{123}I-iomazenil, 400 mg of iodine perchlorate was given orally for thyroid-blockade. Data acquisition started 120 min after injection with a triple-head camera (Prism 3000, Picker, OH, USA) equipped with a cardiac fan-beam collimator, with an acquisition matrix of 128 × 128 pixels, 50 s/view. Iterative reconstruction of the transaxial slices was performed. The transaxial slices were reorientated in a plane that extended from the base of the frontal lobe to the occipital lobe on a mid-sagittal image. Coronal slices were reorientated perpendicular to this plane. All slices were 7.5 mm (three pixels)

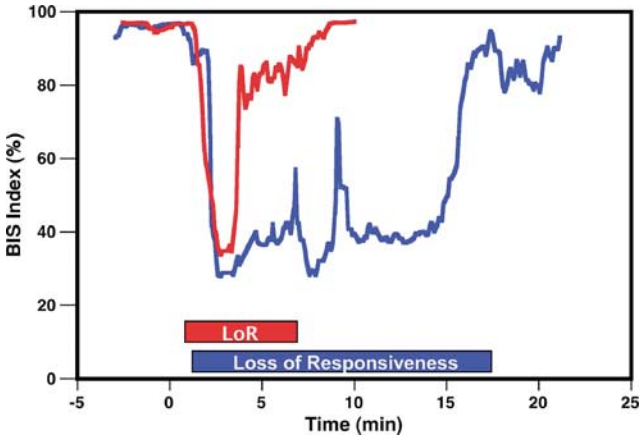

Figure 1 Bispectral indices in the index patient (red line, 0.3 mg etomidate/kg body weight) and a control (blue line, 0.25 mg etomidate/kg body weight). At time point 0 min, etomidate was administered. Interruption of the curves indicates the application of the electrical stimuli aimed at producing a seizure. Horizontal bars indicate the time periods of loss of responsiveness of the index patient and the control to verbal commands. After loss of responsiveness to verbal commands, 0.75 mg succinylcholine/kg body weight was administered.

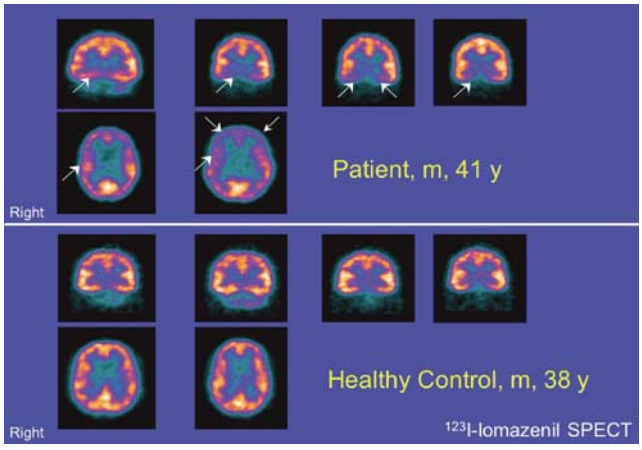

Figure 2 Results of ^{123}I-iomazenil SPECT investigations recorded with a triple-head camera in the index patient and a control. The density of $GABA_A$ receptors is shown in the brain. Light colors indicate a high density and dark colors a low density. Arrows indicate diminished binding in the index patient compared to the control. The four upper pictures are coronal and the two lower pictures are horizontal views of the index patient and the control.

thick. In the index patient, in the frontal and orbitotemporal regions, mainly in the right hemisphere, there is a clearly diminished binding of the radiotracer compared to the control.

Sequences from exons 7 and 8 of the GABRB1, GABRB2, and GABRB3 genes encoding the β1-, β2-, and β3-subunits of the $GABA_A$ receptor, respectively, were amplified from genomic DNA isolated from peripheral blood by polymerase chain reaction using the following primers: UR-120: 5′-TAG GGG TGC TGT GAA AGG AAG AAG A-3′ and UR-121: 5′-GAG AGC CCT TGC CTA TAA TTC CTG ATA-3′ (GABRB1, exon 7); UR-122: 5′-GTG GCA CCT TCA GCT AAG TGT TGT CTT-3′ and UR-123: 5′-GAC TTG GGG TTG AGT TCC AGG GTA TAT TA-3′ (GABRB1, exon 8); UR-111: 5′-CCT ATC CCA GGT TAT CCC TCA GCT-3′ and UR-131: 5′-TCA TGC ACC CCM AAT TTC AGG A-3′ (GABRB2, exon 7); UR-128: 5′-AGA TTG TGG CAA TAT ATG AAT GAG AAA AT-3′ and UR-119: 5′-TGA CAT CCA GGC GCA TCT TCT C-3′ (GABRB2, exon 8); UR-115: 5′-CCT ATC CTC GAC TGT CAC TGA GCT-3′ and UR-133: 5′-AAC TAC AGC CCT TGR ACT CT-3′ (GABRB3, exon 7); UR-130: 5′-ATT CAA CCC CTT ATC TCT GAC TAC TTA AAG-3′ and UR-118: 5′-TTC GCT CTT TGA ACG GTC ATT CTT-3′ (GABRB3, exon 8) and sequenced with an automated sequencer (ABI Prism 310 Genetic Analyzer). This analysis yielded a silent G to A substitution in the third position of amino acid 257 of the β1 subunit.

DISCUSSION

During a course of ECT in a patient suffering from severe generalized anxiety in an episode of major depressive disorder, anesthesia was extremely difficult to induce because of difficulty in assessing the depth of anesthesia clinically. First, unusually high doses of etomidate (up to 1 mg/kg of body weight, standard dose range: 0.15–0.3 mg/kg of body weight) and also, once, propofol (5.6 mg/kg body weight for an anesthesia lasting 35 min, standard dose range: 0.3–4 mg/kg body weight/h) were needed in order to induce satisfactory anesthesia. This patient showed a decreased sensitivity to etomidate and propofol. In studies on recombinant receptors, mutations at amino acid 265 in the second and amino acid 286 in the third transmembrane region of the $GABA_A$ receptor β subunits β1, β2, and β3 have been demonstrated to render $GABA_A$ receptors largely insensitive to etomidate and/or propofol action *in vitro* (Belelli *et al*, 1997; Krasowski *et al*, 1998; Siegwart *et al*, 2002). We therefore sequenced these regions in all three β subunit genes, but were unable to detect a mutation that would cause an amino-acid change. However, we discovered a G to A base substitution in the third position of amino acid 257 of the β1 subunit (ACG ACA GTG instead of the wild-type ACG ACG GTG in amino acid positions 256–258). Both ACA and ACG code for a threonine residue. Therefore, this polymorphism is probably without functional significance. However it is possible that this polymorphism could be related to an altered $GABA_A$ receptor function, since it has been demonstrated that alternative splicing resulting in a mutant version of the protein may be caused by nucleotide changes outside of the splice donor or acceptor sites (Siffert *et al*, 1998).

The use of the BIS bispectral monitor allows a continuous monitoring of the depth of the anesthesia (Rosow and Manberg, 2001), and in this case prompted us to use standard doses of etomidate (0.3 mg etomidate/kg body weight). Based on the clinical assessment of the depth of the hypnotic state in this patient alone, a successful induction of anesthesia at standard doses of the anesthetic agents would not have been feasible.

In a ^{123}I-iomazenil SPECT study we found in this patient substantially diminished binding of the radiotracer to benzodiazepine receptors in the frontal and orbitotemporal regions compared to the control subject, with a predominance of the defect in the right hemisphere. This result can be best explained by the coexisting anxiety disorder, since there are no comparable results available in depressed patients (Nutt and Malizia, 2001). Our reported findings do not seem to be related to the effect of ECT, since in an earlier study the iomazenil-binding capacity significantly increased 7 days after a mean of seven ECT sessions compared to a baseline value registered before the ECT course bilaterally in the frontal, parietal, and occipital regions as well as in the right prefrontal area (Mervaala *et al*, 2001). The apparently decreased binding capacity of $GABA_A$ receptors seems to be related to the clinically observable decrease in sensitivity to the anesthetic agents.

This case illustrates how clinical observations in psychiatry can prompt investigations by modern techniques and potentially link clinics and basic sciences and add to the understanding of physiopathology. The clinical, genetic, and functional imaging findings, in this patient suffering from depression and anxiety, taken together are interesting and possibly linked to *in vivo* $GABA_A$ receptor function. To our knowledge, this is the first report of a link between insensitivity to anesthetic agents and altered $GABA_A$ receptor function in a clinical case. However, no definitive conclusions can be made about causal links in this single case. Whether these findings are linked to the diagnosis of anxiety or depression is a matter of speculation. To our knowledge, such associations have not yet been investigated in depressed patients.

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