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Specific binding of [^{3}H]Ro 19-6327 (lazabemide) to monoamine oxidase B is increased in frontal cortex of suicide victims after controlling for age at death ☆

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Abstract Previous studies have reported negative findings for the association among brain monoamine oxidase B (MAO-B) and suicidal behaviour. However those studies did not adequately control their main results for the influence of confounding variables such as age at death. We have evaluated the association of MAO-B density (assessed by [^{3}H]Ro 19-6327 – lazabemide – binding) with type of death (suicide victims *vs* non-suicide controls) after controlling for age at death. Frontal cortex samples from 43 subjects (21 suicides, 22 controls) were assayed for MAO-B density at a single concentration of lazabemide (8 nM). A linear regression modelling approach comparing nested models resulted with both type of death ($p<0.05$) and age of death ($p<0.01$) as main explanatory variables for the variability of MAO-B density. Suicide victims had >30% more binding sites for lazabemide than controls. Contrary to previous reports, MAO-B density seems to increase in suicide victims.

1. Introduction

The monoamine oxidase B enzyme (EC 1.4.3.4) (MAO-B) plays a major role in the inactivation of monoamine neurotransmitters, in the pathophysiological generation of cytotoxic free radicals during aging, and in neurodegenerative disorders (Youdim, 2006). In the human brainstem MAO-B is expressed by serotonergic neurones which allows the enzyme to be an excellent marker for serotonergic innervation (Saura et al., 1996). In other brain areas, MAO-B is also expressed by histaminergic neurons and astrocytes (Saura et al., 1996). Evidence for deficiencies of brain serotonin neurotransmission in suicidal behaviour and depression is well established (Stockmeier, 2003). More recently, neuronal and glial cell atrophy and loss have been observed in postmortem cortical brain areas of subjects with mood disorder (Manji et al., 2001; Miguel-Hidalgo and Rajkowska, 2003). Therefore, alterations of the MAO-B enzyme could underlie some aspects of the pathophysiology of suicide and/or affective

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disorders. Specifically, abnormalities in brain MAO-B activity have been considered instrumental to suicidal behaviour and affective disorders but, so far, mostly negative results have appeared in the literature. We know of five previous reports which have analysed the MAO-B activity, or density, in the frontal cortex of suicide victims and control subjects (Grote et al., 1974; Gottfries et al., 1975; Mann and Stanley, 1984; Sherif et al., 1991; Sastre and García-Sevilla, 1997). And one of them (Grote et al., 1974) could be dismissed because of using kynuramine as substrate to evaluate MAO activity and, therefore, that study assessed additionally the activity of the MAO-A isoenzyme (Naoi et al., 1987).

Due to the conflicting results reported in those studies and the availability of [^{3}H]Ro 19-6327 (lazabemide) to study MAO-B density, we decided to carry out a postmortem study of a group of suicide victims and a group of non-suicide controls to further clarify the biological status of the MAO-B enzyme in suicidal behaviour. In addition to match, at the design stage, suicide victims and controls by age at death and other confounding variables, we decided to use, at the analyses stage, a modelling approach with appropriate linear regression models to explain the variability of MAO-B density.

2. Methods

2.1. Subjects

Frontal cortex brain samples (Brodmann's area 9) were obtained at autopsy at the Instituto Vasco de Medicina Legal (Bilbao, Spain) from 43 subjects (21 suicide victims and 22 controls). All deaths were mainly violent and by sudden mechanisms in both groups. The causes of death in the suicide group were hanging (11 subjects), jumping from height (8) and intoxication (2); whereas those in the control group were traffic and car accidents (9), sudden death (4), work accidents (3), accidental fall (2), pneumonia (1), traumatic accident by bull running (1), accidental intoxication (1) and drowning (1). A careful retrospective search for psychiatric diagnoses was done for those suicide victims who had former contacts with psychiatric facilities as recorded in Coroners' reports. Because alcoholism has been associated with alterations of MAO-B activity in human brain (Gottfries et al., 1975), we also obtained information about its presence as previously described (Maeztu et al., 1997). Slightly over a half of suicide victims (n=11, 52%) had a confirmed psychiatric diagnosis other than alcoholism, before or at the suicidal time (6 subjects were diagnosed of depressive disorder, 2 of anxiety disorder, 1 had bipolar type I disorder, 1 schizophrenia and 1 was diagnosed of psychotic disorder). We cannot rule out the presence of psychiatric disorders in the rest of suicide victims because psychological autopsies with relatives were not routinely performed. Control subjects, with no known psychiatric or neurological disease except for alcoholism, were obtained and matched to suicide victims as closely as possible according to sex, age at death, and postmortem delay. Toxicological screening for blood alcohol or psychotropic drugs was also obtained when available. Unfortunately no information was recorded about tobacco consumption.

All the experiments and procedures used for clinical data extraction were approved by the appropriate ethic and research committees and were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.2. Procedures

Frontal cortex brain samples were dissected at autopsy and immediately frozen and stored at −70 °C until assays. After thawing, neural membranes (P_2 fractions) were prepared as previously described (Meana et al., 1992). The tissue samples were homogenized in 5 ml of ice-cold Tris–sucrose buffer (5 mM Tris–HCl, 250 mM sucrose, and 1 mM $MgCl_2$; pH 7.4). The homogenates were then centrifuged at 1100 ×g for 10 min, and the supernatants recentrifuged at 40,000 ×g for 15 min. The pellet so obtained was washed twice with 2 ml of fresh incubation buffer (50 mM Tris–HCl, 130 mM NaCl, 5 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 5 mM EGTA; pH 7.4), recentrifuged at 40,000 ×g, and resuspended in an appropriate volume of incubation buffer. Protein contents were determined with bovine serum albumin as standard. The protein values (mean ± SD) were similar in suicide victims (1.53 ± 0.56 mg/ml) and control subjects (1.51 ± 0.49 mg/ml).

Total binding was measured in 0.5 ml aliquots of neural membranes which were incubated in triplicate for 90 min at 22 °C with a single concentration of [^{3}H]Ro 19-6327 (8 nM), close to the dissociation constant values previously reported (Da Prada et al., 1990; Saura et al., 1992; Sastre and García-Sevilla, 1993). Nonspecific binding was assessed in the presence of the MAO-B selective inhibitor (−)-deprenyl (10^{-4} M). The specific binding of [^{3}H]Ro 19-6327 to brain MAO-B membranes was defined as the difference between total and non-specific binding. Incubations were finished by diluting the samples with 5 ml of ice-cold incubation buffer (4 °C). Membrane-bound [^{3}H]Ro 19-6327 was separated by rapid filtration under vacuum through Whatman GF/C glass fiber filters which had been presoaked with 0.5% polyethylenimine. The filters were rinsed twice with incubation buffer and transferred to minivials for radioactivity counts by liquid scintillation spectrometry.

2.3. Drugs and chemicals

[^{3}H]Ro 19-6327 (specific activity: 20.2 Ci/mmol) was generously obtained from Dr. J.G. Richards and Dr. J. Saura (F. Hoffman, La

Table 1 Main characteristics of suicide victims (n=21) and controls (n=22)

Variable	Suicides	Controls	p value [a]
Sex			
Males	17	18	
Females	4	4	1.0
Age at death (years)	51 ± 12	53 ± 12	0.54
Alcohol abuse/alcoholism			
Yes	12	8	
No	9	14	0.23
Blood alcohol levels			
Yes (>0)	9	9	
No (=0)	9	6	
Not available	3	7	0.40
Blood screening for psychotropic drugs			
Positive	7	0	
Negative	10	15	
Not available	4	7	0.011
Postmortem delay [b] (h)	45 ± 23	46 ± 25	0.91
Storage time at −70 °C [c] (months)	22 ± 20	13 ± 11	0.07

Values are expressed as frequencies or as means ± SD.
[a] P values according to Fisher's exact test or t-test.
[b] Time between death and autopsy.
[c] Time between freezing frontal cortex samples and experiments.