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Variations in respiratory distress characterize the acute agonal period during heroin overdose death: Relevance to postmortem mRNA studies

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Abstract

Aims: To determine whether there are factors during apparent rapid heroin overdose death that affect agonal state and thus brain pH (index of hypoxia) that can influence neurobiological systems linked to drug abuse.
Design and methods: Brain specimens and autopsy/medical reports were investigated in subjects dying from heroin overdose ($n = 70$) and compared to normal controls ($n = 45$) as well as suicide victims ($n = 31$) with a documented rapid cause of death. Detailed autopsy material was characterized as to positive and negative respiratory distress in relation to brain pH; drug toxicity and other demographic information was also evaluated. In situ hybridization histochemistry was used to study mRNA expression levels of dopamine (e.g., D2 receptor, dopamine transporter) and opioid (e.g., proenkephalin) related markers in various structures in relation to brain pH.
Findings: Brain pH was generally reduced in heroin overdose cases versus normal and suicide subjects. There was, however, significant variation in heroin overdose deaths related to differences in respiratory distress that differentially altered brain pH levels. Various factors such as vomit inhalation, resuscitation, pulmonary embolism and suffocation contributed to positive respiratory distress. Elevated brain pH was observed in heroin overdose with positive alcohol toxicity suggesting potentiated alcohol-induced rapidity of heroin deaths. mRNA expression levels of the dopamine-related genes and proenkephalin were positively correlated with brain pH.
Conclusions: Respiratory distress contributes to variations in the acute agonal state during heroin overdose death that differentially alters brain pH levels and significantly impacts mRNA levels. Such findings should be considered for postmortem molecular/neurochemical neurobiological studies of opiate abusers.

Keywords: Suicide; Brain pH; Opiate abuse; Dopamine; Opioid neuropeptide

1. Introduction

Opiate and, in particular, heroin use is widespread worldwide and heroin overdose (OD) is one of the major causes of morbidity [37] and mortality [4,7,11,16,30] among active heroin abusers. Despite the use of opiates for thousands of years and the prevalence of heroin abuse in society there is still relatively little known about the underlying neurobiology of heroin addiction. An important research strategy in recent years in attempts to understand the neurobiology of drug abuse has been the postmortem study of human substance abusers. There were initially reasonable concerns that such studies would be unfeasible due to factors at death or after death that would compromise the intactness of proteins, DNA or mRNA in human postmortem tissue. A number of studies have addressed these issues and several pre- and postmortem factors influencing the outcome of human postmortem experiments have now been recognized [8,19,29]. Postmortem interval (PMI) was postulated to be one of the most crucial factors influencing the integrity of molecules [39], but today it is well recognized that PMI has only a relatively modest impact [1,21,23,32,36].

Agonal state or the premortem phase has also been identified to contribute to the diversity of mRNA, protein or enzyme

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activity levels in postmortem human brain [3,13,14,20,36,40]. Agonal state incorporates the events happening around the time of death and is very dependent on the cause and rapidity of death. According to several reports agonal state is closely related to brain pH [12,20,25,40]. Based on the fact that the decrease in brain pH is most evident in prolonged periods of serious illness (e.g., pneumonia, cancer and cerebrovascular disease), pH has been proposed as a marker of protracted agonal period. Studies examining the association between brain pH, agonal state and mRNA levels have almost exclusively examined subjects with diverse neuropsychiatric conditions such as Alzheimer's disease, dementia, Parkinson's disease, major depression or bipolar disorder, which often are associated with long agonal states. Very few studies to date have examined neuropsychiatric conditions with apparent short agonal periods.

In initial phases of our investigation of the molecular neurobiology of heroin abusers who died from OD, we noted significant variations in brain pH. The current study was thus carried out to determine the conditions in heroin OD, which is considered a rapid cause of death that might contribute to alteration of brain pH and thus impact mRNA levels. To examine closer the interaction between short agonal state and brain pH, in addition to normal controls, we examined suicide subjects with a documented rapid cause of death. The results revealed that heroin OD cases with evidence of respiratory distress have reduced brain pH that significantly contribute to reduced mRNA levels of several genes relevant to the neurobiology of drug abuse.

2. Materials and methods

2.1. Tissue collection and subject characterization

Human brains, 227, were collected from January 1995 until December 2004 with a postmortem range of 4–48 h. From all the investigated cases with available pH values, 146 cases were retrospectively selected for this study. The majority of the human brain specimens ($n = 126$) was obtained at autopsy at the Department of Forensic Medicine at Semmelweis University (Hungary) under the guidelines approved by the Semmelweis University Human Ethical Committee. Other cases ($n = 20$) were obtained from the National Institute of Forensic Medicine (Karolinska Institutet, Stockholm, Sweden) under the guidelines approved by the Ethics Committee at Karolinska Institutet and the Swedish Board of Social Welfare. Medicolegal investigations of the deaths were conducted by forensic pathologists. The cause and manner of death were determined after evaluating the circumstances of death (e.g. respiratory distress; see below), toxicology data, and autopsy results. All cases were evaluated for common drugs of abuse (including alcohol) and also for therapeutic drugs [9].

Subjects were classified into three main groups according to their cause of death: controls, heroin OD and suicide subjects. The control group consisted of cases with diverse causes of death presented in detail in Table 1. The heroin group consisted of pure heroin OD ($n = 61$) as well as heroin OD cases with additional positive toxicology for other substances (THC, $n = 5$; benzodiazepine, $n = 3$; amphetamine, $n = 1$). Based on information when available about the decedent's past drug history, most subjects had only approximately 2 years heroin use and no methadone, naltrexone or buprenorphine treatment. For the purposes of the study, the suicide group consisted mainly of hanging which is known to result in a very rapid death; jumping from high places was also included. Table 1 presents the demographic data of all subjects.

The autopsy reports and any other available reports (hospital and ambulance records) of all the cases were evaluated. Blinded to the information regarding the pH values, conditions which might cause hypoxia, lactate acidosis and a consequential drop in pH were termed "respiratory distress". This term was first used in connection with brain pH [21] to describe the state when the organism due to oxygen deprivation starts anaerobic glycolysis and the accumulating lactate causes low pH. Conditions included in the respiratory distress group were: gross and microscopic evidence of vomit inhalation, gross and microscopic evidence of bronchopneumonia or other pneumonia, septic state, pulmonary embolia, suffocation and resuscitation.

2.2. pH measurement

Routine pH measurements were conducted for 146 cases which fit the criteria of the study. A piece of tissue (0.9–1.4 g) from frozen cerebellar cortex was homogenized in 10 volumes of double distilled water (pH has been adjusted to 7.0). The homogenates were centrifuged at 4 °C at maximum speed for 20 min. The supernatants were subsequently maintained at room temperature for 15 min and pH was measured (PHM 92 LAB pH meter, Radiometer, Copenhagen, Denmark) in triplicates.

2.3. In situ hybridization histochemistry

Coronal sections (20 μm-thick) were taken from the brainstem and striatal blocks of a subset of postmortem brain material using a Jung-Frigocut 2800E cryostat (Leica, Heidelberg, Germany) and thaw-mounted onto poly-l-lysine glass slides and stored at −30 °C. Riboprobes complementary to various human genes including the tyrosine hydroxylase, dopamine D2, and preproenkephalin genes were prepared by in vitro transcription. The RNA probe for the dopamine D2 receptor was synthesized from a full length 1.58 kb cDNA fragment of the gene for the long form of the human receptor ([10]; provided by Dr. O. Crivelli), subcloned into a pBSKS plasmid vector; the D2 probes were reduced by alkaline hydrolysis to increase penetrance. The RNA probe for the tyrosine hydroxylase (TH) was generated from DNA template corresponding to nucleotides 766–1079 of TH gene (GenBank accession No. NM_000360). The proenkephalin probe was a *Eco*RI/Pvu 792 bp fragment complimentary to the full coding region of the preproenkephalin human gene that was subcloned in a psp65 plasmid [26]. Riboprobes complementary to the human DAT mRNA were synthesized from human cDNA *Bam*H1/*Sac*I 809 bp fragment subcloned into a pSP73 plasmid [17].

The in situ hybridization histochemistry experiments were optimized for each probe based on already published protocols [18]. Briefly, the labeled probe was applied to the brain section in a concentration of 2×10^3 cpm/mm^2 of coverslip area. Two adjacent sections from each subject were studied. Hybridization was carried out overnight at 55 °C in a humidified chamber. After in situ hybridization, the slides were apposed to Hyperfilm. The mRNA expression level was estimated by densitometric readings within specific brain structures of interest.

2.4. Statistical analyses

The mean of three pH measurements per subject was used for the statistical analyses. Univariate analyses were carried out to obtain an overall estimate of the general characteristics of each individual variable on the brain pH levels. General Linear model was used to determine the effect of group (control, suicide and heroin OD) and the impact of different variables (e.g., age, gender, PMI, storage time and respiratory distress) on brain pH. The variables with the weakest effect on the model were sequentially deleted and only those with a $p < 0.05$ association with brain pH were included in the final statistical model as covariates. Post hoc group effects were evaluated by Tukey–Kramer analyses. Pearson correlation was used to determine the relationship between brain pH with blood/urine toxicological measurements. Statistical analyses were carried out using JMP (5.1) software package (SAS Institute, Inc., Cary, NC, USA). Statistical significance was set as $p < 0.05$ and trends considered for $p < 0.10$.

3. Results

Fig. 1 shows the mean pH values in three groups of subjects. The overall pH values from 146 subjects ranged between 6.06 and 7.16 (6.69 ± 0.019). There was an overall significant main

Table 1
Demographical data of the subjects

Group	Age (years)	Gender	PMI (h)	ST(weeks)	COD	Inclusion criteria
Control $N=45$	36.07 ± 1.96, (15–65)	M = 35 F = 10	20.57 ± 0.99	183.6 ± 16.8 (44.1–373.1)	Accidental falling down ($n=1$), alcohol intoxication ($n=4$), electric shock ($n=4$), pulmonary embolia ($n=2$), GI tract perforation ($n=1$), myocardial infarct ($n=24$), pneumonia ($n=3$), stabbing (homicide) ($n=1$), accidental suffocation ($n=1$), sudden death ($n=4$)	Negative toxicology of opiate or other drug No history of opiate abuse No history of abuse to other drugs No needle tracks
Heroin OD $N=70$	25.82 ± 0.67, (17–46)	M = 57 F = 13	23.92 ± 0.35	223.8 ± 18.2 (60.8–548)	Heroin OD	Positive opiate toxicology History of opiate abuse Needle tracks
Suicide $N=31$	35.9 ± 2.53, (15–81)	M = 22 F = 9	23.74 ± 1.13	255.5 ± 26.2 (88.1–527.7)	Suicide by hanging ($n=29$) and jumping ($n=2$)	Negative toxicology of opiate or other drug No history of opiate abuse No needle tracks History of depression, suicide attempts and statements

Exclusion criteria: head trauma, positive HIV status. Values presented as mean ± S.E.M., range in parenthesis. Abbreviations: PMI, postmortem interval; ST, storage time; COD, cause of death; M, male; F, female; y, years; h, hour; OD, overdose; GI, gastro-intestinal.

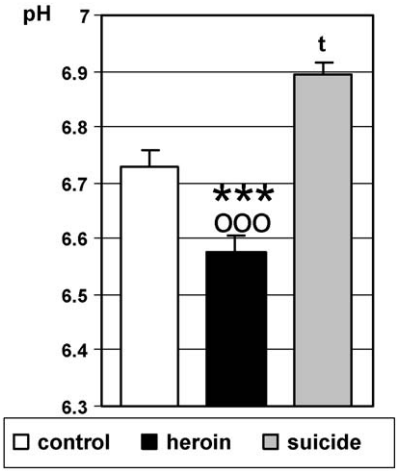

Fig. 1. Means and standard errors of pH values measured in the brains of control, heroin overdose and suicide subjects. Overall group effect ($F_{6,144} = 23.85$, $p < 0.001$) covaried for respiratory distress and alcohol toxicity $^{***}p < 0.001$ vs. control; $^{ooo}p < 0.01$ vs. suicide; $t = p = 0.0639$ vs. control.

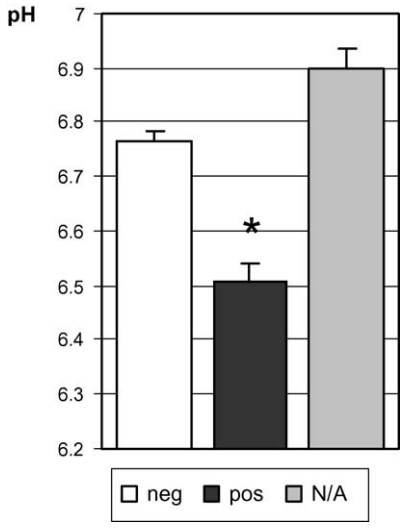

Fig. 3. Mean brain pH values in correlation with respiratory distress. negative–negative respiratory distress ($n = 85$), positive–positive respiratory distress ($n = 49$), N/A—data not available regarding respiratory distress ($n = 12$). $^{*}p < 0.05$ vs. control.

effect of group (ANCOVA $F_{6,144} = 23.848$, $p < 0.0001$; covaried for respiratory distress and ethanol toxicity as detailed below).

The distribution of pH values within each group is presented in Fig. 2. In the control group, pH levels ranged between 6.15 and 6.98 (6.72 ± 0.028). The pH values in the heroin group (6.57 ± 0.025) contributed to the wide variation of the total population; the highest and lowest pH measurements were from this group. In the suicide group, the range of pH was between 6.41 and 7.08 (6.89 ± 0.024). Post hoc statistical analyses showed that the heroin group had significantly lower pH values than the control and suicide groups ($p < 0.0003$). There was a non-significant trend ($p = 0.0639$) for brain pH to be higher in the suicide group as compared to controls. Based on the significant group differences in brain pH, all possibly contributing variables were examined separately in each group.

Univariate analyses showed no significant effect of age, gender, PMI or brain storage time on the brain pH levels. However, respiratory distress and ethanol toxicity were found to significantly influence the brain pH levels. Detailed description is provided below regarding the impact of these variables on brain pH.

3.1. Respiratory distress

The presence or absence of respiratory distress was defined as "positive respiratory distress" or "negative respiratory distress", respectively. Positive respiratory distress correlated with lower pH values in comparison to negative respiratory distress when measured in all examined groups (negative respiratory distress, pH 6.77 ± 0.018; positive respiratory distress, pH 6.59 ± 0.03, $p < 0.0001$; Fig. 3).

Evaluation of the respiratory distress in each group revealed that the majority of subjects contributing to the positive respiratory distress were within the control and heroin groups. In the control group, all subjects with low pH (<6.5) were only present in the positive respiratory distress (Fig. 4A). In contrast, 16.6% of heroin subjects with no documentation of respiratory distress had low brain pH (Fig. 4B).

In the suicide group, there was a narrow range of brain pH in the negative respiratory distress group between 6.73–7.08 (6.91 ± 0.021). The lack of respiratory distress was associated

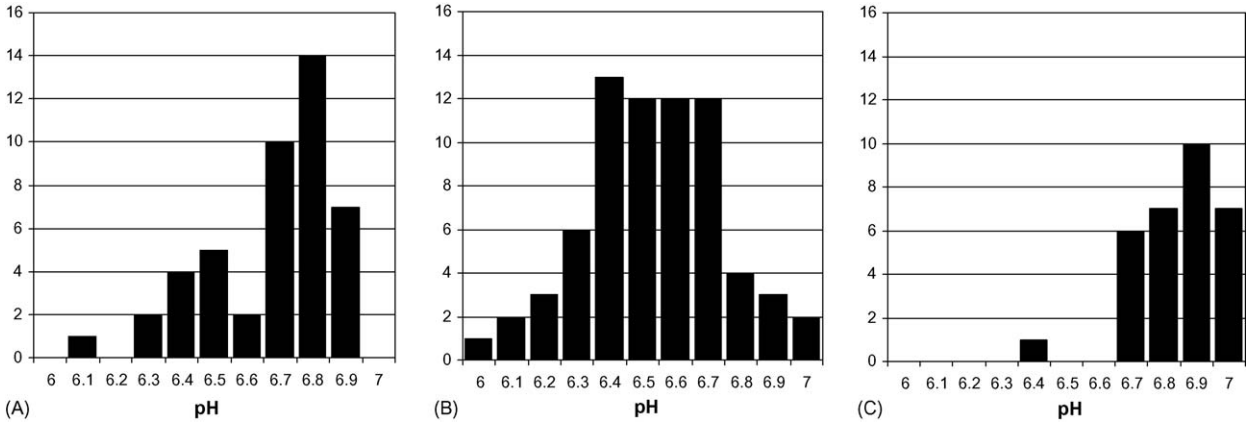

Fig. 2. Distribution of pH values in control (A), heroin overdose (B) and suicide (C) subjects. The *y* axis represents the number of cases at each pH value.

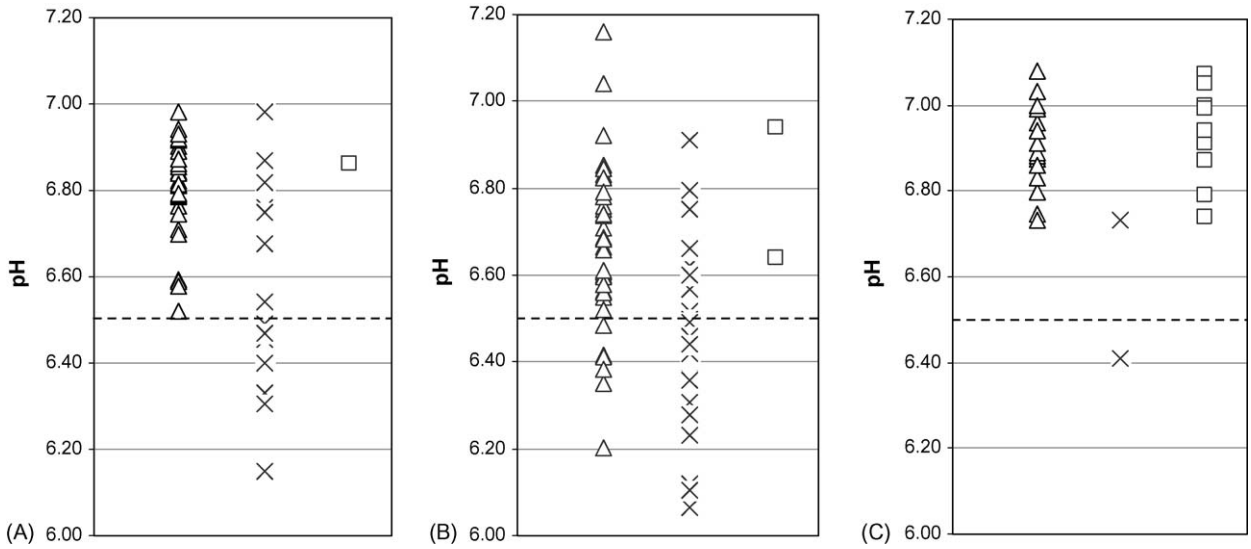

Fig. 4. Brain pH levels in relation to respiratory distress in control (A), heroin overdose (B) and suicide (C) groups. The triangles represent negative respiratory distress cases, the crosses represent positive respiratory distress cases and squares depict results with undetermined respiratory distress. The dotted line shows the average brain pH values in association with positive respiratory distress.

with a significant increase of pH ($p = 0.0006$). There was also a group of nine subjects with undefined respiratory distress with pH 6.93 ± 0.037, values consistent with the negative respiratory distress group. Only two subjects belonged to the positive respiratory distress group; both were resuscitated previous to death and both had low pH values (Fig. 4C).

3.2. The effect of heroin metabolites on brain pH

Heroin metabolites were present only in the heroin group. The metabolites screened in association with autopsy were 6-monoacetylmorphine (6-MAM), morphine and codeine in blood and urine. As 6-MAM was present in the blood or urine in only a small number of cases ($n = 9$), this opiate metabolite was not included as a parameter in the statistical analyses. There was no correlation between blood morphine concentration and brain pH. In the univariate analysis, urine morphine concentration had a significant relationship with brain pH ($p = 0.0260$). There was a weak, but significant correlation between brain pH and urine morphine concentration ($r = -0.3194$, $p = 0.0237$). This significance was however due to two subjects with high morphine values (34.49 and 19.96 μg/ml). There was no significant correlation when the two subjects were excluded ($r = 0.0002$; $p = 0.9989$). No correlation was evident between brain pH and codeine concentration in the blood or urine.

3.3. The effect of ethanol on brain pH

The univariate analyses carried out on all the subjects showed that urine and blood ethanol levels had a significant effect on brain pH ($p = 0.0490$ for urine ethanol; $p = 0.0207$ for blood ethanol).

In the heroin group, blood and urine ethanol concentrations significantly influenced brain pH levels (blood ETOH $p = 0.0022$, urine ETOH $p = 0.0049$). As the concentration of ethanol in urine and blood were very highly correlated ($r = 0.9624$, $p < 0.0001$) and the significance for the blood ethanol was stronger than for urine ethanol, further statistical analyses were performed only with blood ethanol concentration levels. Inclusion of blood ethanol concentration as a covariant in the statistical model revealed that subjects with positive respiratory distress still had significantly lower pH values than individuals with negative respiratory distress ($p = 0.0003$, covaried for blood ethanol concentration $p = 0.0067$). When the negative and positive respiratory distress groups were analyzed separately, it was observed that the presence of ethanol in blood in the negative respiratory distress group was significantly associated with brain pH ($p = 0.0083$). Analyses of the heroin subjects showed significant correlations between brain pH and blood ($r = 0.4346$, $p = 0.0091$) as well as urine ($r = 0.4656$, $p = 0.0048$) ethanol levels in the negative respiratory distress group. There was no such association in the positive respiratory distress group with ethanol toxicity ($p = 0.6271$) (Fig. 5).

Neither in the control nor in the suicide group was there any correlation between the blood or urine ethanol concentrations and brain pH.

3.4. Brain pH effects on mRNA levels

The mRNA levels of genes relevant to the neurobiology of drug abuse were examined in the brainstem and striatum of a subset of control and heroin subjects. The genes of interest were associated with the dopamine (e.g., tyrosine hydroxylase, dopamine D2 receptor, dopamine transporter) and opioid (preproenkephalin) systems that are strongly linked with the neurobiology of drug abuse [22]. Consistent with previous findings [17] the mRNA expression pattern for the dopamine markers were very similar with highest levels in the paranigral nucleus of the ventral tegmental area and in the ventral division of the sub-

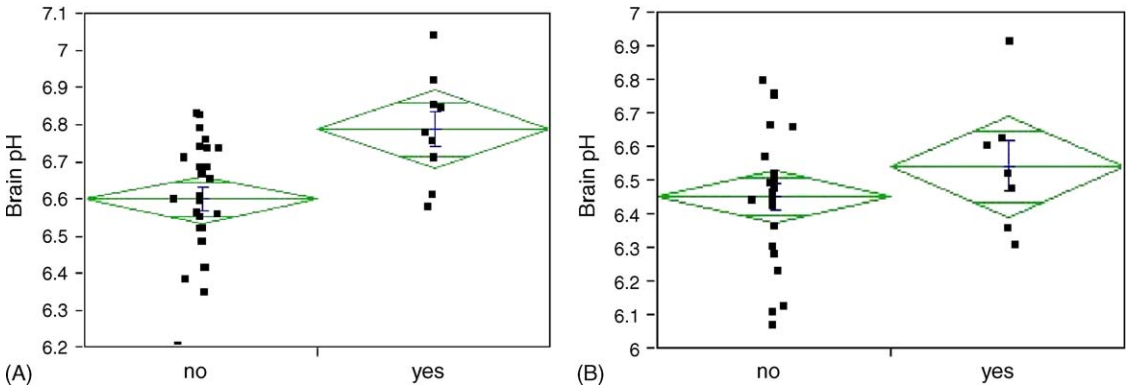

Fig. 5. Ethanol use in relation to brain pH levels in negative (A) and positive (B) respiratory distress groups. (no, no toxicological evidence of ethanol use; yes, positive ethanol toxicology).

stantia nigra. The results showed that subjects with low brain pH, irrespective if a control subject or heroin user, tended to have low mRNA expression levels especially evident in the high mRNA expressing regions (Fig. 6). For example in the brainstem, the paranigral nucleus showed a significant decrease of dopamine D2 receptor mRNA expression in correlation with reduced pH levels ($r = 0.466$, $p = 0.0094$) with a trend effect in the substantia nigra pars ventralis ($r = 0.34$, $p = 0.0569$). There was a similar positive correlation between pH levels and the dopamine transporter mRNA expression levels (paranigral nucleus: $r = 0.514$, $p = 0.0037$; substantia nigra pars ventralis: $r = 0.417$, $p = 0.022$). A similar pattern was also evident for the tyrosine hydroxylase expression levels. A significant decrease was also observed in the striatum for preproenkephalin mRNA expression in relation to pH levels; significance most evident in the putamen and nucleus accumbens core ($r = 0.409$, $p = 0.0086$; $r = 0.37$, $p = 0.0203$).

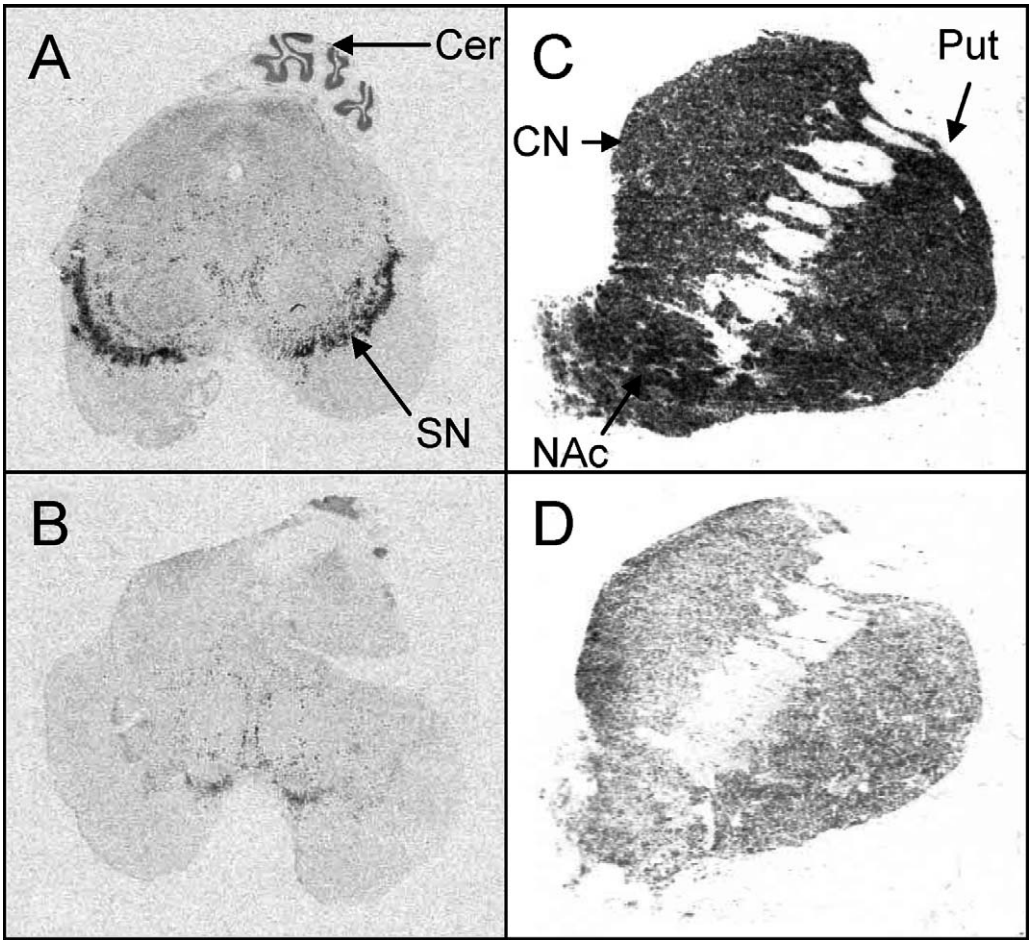

Fig. 6. Autoradiogram images showing the mRNA expression of the dopamine D_2 receptor in the human midbrain (A and B) and preproenkephalin in the striatum (C and D) in subjects with high (A and C) and low (B) brain pH. Cer, cerebellum; SN, substantia nigra; CN, caudate nucleus; Put, putamen; NAc, nucleus accumbens.

4. Discussion

The current investigation shows that apparent rapid heroin OD cases have variable brain pH levels associated with different acute agonal states even around the time of death that significantly impacts mRNA levels central to molecular postmortem studies of opiate abuse.

The brain pH values measured in this study were comparable to previously published results although our values tended to be higher than some reports due to our focus on suicide cases with rapid death. The suicide group was included in the present investigation since we had hypothesized that suicide by hanging and jumping, which are extremely fast manners of death, would not allow time for prolonged terminal hypoxia. As such, the pH values should be the highest in the suicide victims. This hypothesis proved to be valid considering that the suicide group had the highest pH levels, to a much greater extent than the "rapid" death normally though to occur in heroin OD. As expected, the normal control subjects had lower mean pH than the suicide group, but still higher than the heroin OD cases. The normal control group generally consisted of subjects with variable causes of death, from electric shock, which is known to cause instantaneous death, to subjects with pneumonia that results in low pH. Moreover, only 29% of the controls had evidence of positive respiratory distress as compared to 44% in the heroin group which contributed to higher brain pH in the control group as compared to the heroin subjects. The fact that the heroin OD group had the lowest mean pH value is probably attributed to the higher number of positive respiratory distress cases which is discussed in detail below. However, it is important to note that morphine, as all the opiates, causes respiratory depression which in turn can lead to hypoxia and impact brain pH. Respiratory depression to date cannot be evaluated postmortem, thus such cases could be falsely included in the negative respiratory distress group of heroin subjects who had no forensic evidence of respiratory problem, but had low brain pH.

Many attempts have been made to classify agonal state [12,20,25,27,29,36]. The reports usually segregate death cases into two major groups: death without and with preceding disease. The first group is often subdivided into violent and natural death cases. The subdivision of the second group is dependent on the severity and duration of the disease and the presence of coma or assisted ventilation in the terminal phase. According to these scales all of our subjects would be included in the first group which contains rapid deaths of violent or natural origin. The fact that pH values varied within the current population of subjects emphasize that there is still a significant degree of heterogeneity even in subjects without slow deaths. This diversity was particularly evident in the heroin OD group. In the present study, dissociating subjects in regard to respiratory distress (modified from Kingsbury et al.) helped in part to provide a more systematic means of evaluating the influence of events during the relatively short agonal time period generally characteristic of heroin OD.

Some of the long-term chronic conditions included in the respiratory distress group such as pneumonia or sepsis have already been accepted as factors contributing to hypoxia and thus pH reduction [12,29,36]. We could now also identify other respiratory distress factors, such as vomit inhalation, resuscitation, pulmonary embolism, and suffocation, that affected brain pH. Vomit inhalation or aspiration of the acidic content of the stomach, is a very common complication when the coughing reflexes are diminished, as is the case in opiate overdose [2]. It is also documented that morphine, next to its analgesic and euphoric features, has a pro-emetic quality that can induce vomit inhalation. According to forensic sources [15] a considerable number of heroin overdose subjects die of vomit inhalation which is consistent with the high percentage of respiratory distress detected in the current heroin population. In regards to the impact of artificial resuscitation on brain pH**,** it is well known that the ventilation and compression of the chest during this procedure is not entirely sufficient to ensure the proper oxygenation of the organs, and the initiation of anaerobic glycolysis under these conditions is likely to lead to acidosis [35]. In the present study, the influence of resuscitation on brain pH was best evident in the suicide group which had a more homogenous cause of death; the only two suicide subjects with low pH values were attempted resuscitation cases. Pulmonary emboli that blocks the pulmonary arteries, was also included in the respiratory distress category since it decreases the amount of blood being oxygenized in the lungs which can lead to hypoxia and further acidosis. In our study, cases of pulmonary embolism were recorded only in the control group. That was also not unexpected that conditions of suffocation contributed to lower brain pH as that clearly involves hypoxia leading to death. It is important though to distinguish suffocation (which involves a prolonged episode of hypoxia) from hanging where the mechanism of death may involve the compression of the carotid arteries supplying the brain and the loss of consciousness in a matter of 5–10 s after which death usually occurs within 3–5 min. [6]. The fact that brain pH was very high in the suicide hanging cases confirms that rapid death was not a common feature of heroin overdose cases that were characterized by low pH.

Of the other variables studied, no overall correlation was found between brain pH and PMI, brain storage time, gender or age, findings consistent with earlier reports [1,13,29]. There was also no significant association of morphine or its metabolites with pH except with the inclusion of two subjects who had high morphine levels. The concentration of morphine in the body at the time of death has not been a good forensic marker for identifying heroin OD cases [5,33,38] thus it is perhaps not surprising that morphine levels did not relate to apparent agonal state. There was, however, a trend for the ethanol concentrations in the blood and urine to be correlated with elevated pH in heroin subjects who had no evidence of respiratory distress. Fatal heroin OD has often been found to involve ethanol consumption [5,24,28,31]. The mechanism of the interaction between opiates and alcohol is still not clearly understood, but there appears to be a synergistic effect of these compounds such that ethanol potentiates the effect of opiates. A more rapid death would thus lead to the trend for higher brain pH levels in the postmortem brain tissue of heroin subjects with positive ethanol toxicity.

A number of studies have already emphasized the importance of agonal state and brain pH on mRNA expression levels, primar-

ily in long-term pathological cases [19,20,25,29,36]. The current study confirms that reduced mRNA expression contributes to low brain pH and not just due to chronic disease. Brain pH significantly reduced mRNA levels of drug abuse-related genes in heroin cases emphasizing the rapid impact of certain conditions around death on molecular events. Such events might contribute to some of the variability that is frequently evident in postmortem studies that do not relate to PMI or other demographic features of the subject. We also noted a major impact of brain pH on a wide variety of other genes linked to diverse neural functions than dopamine and opioid systems (unpublished results). Thus far, studies to date on, e.g., cocaine-related deaths have not reported significant effects on brain pH [34]. Of the drugs of abuse, heroin users may be most vulnerable to respiratory distress due to its effects to induce respiratory depression and emesis, thus attention needs to be taken when evaluating such cases in postmortem studies.

In summary, the current data suggest that in addition to the cause of death, short agonal state and the presence of the respiratory distress should be taken into consideration in postmortem mRNA studies especially for opiate abusers. It is essential that heroin-related deaths are not only defined by toxicology for identifying supposed OD fatalities, but are also characterized based on forensic evaluation of factors such as respiratory distress since it is a common feature of heroin OD. Brain pH is an effective marker for respiratory distress conditions even during apparent rapid death.

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