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Catecholamine and MHPG Plasma Levels, Platelet MAO Activity, and ^{3}H-Imipramine Binding in Heroin and Cocaine Addicts

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Abstract

This work evaluated in a population of heroin and heroin plus cocaine human addicts:

1. Norepinephrine (NE), epinephrine (Epi), and 3-methoxy-4-hydroxyphenylglycol (MHPG) (the principal metabolite of brain NE) plasma levels;
2. Monoamine oxidase (MAO) activity; and
3. ^{3}H-imipramine specific binding to the amine carrier in platelets.

NE plasma levels were significantly lower in the short-term heroin user groups (1–3 and 4–6 yr), a finding not observed in both the long-term heroin user (>6 yr) and heroin plus cocaine user (>6 yr) groups. Epi levels changed in a similar manner, except that a significant increase was noted in heroin plus cocaine abusers. Conversely, dopamine and MHPG plasma levels increased with the duration of heroin use, and even more with cocaine abuse. Platelet MAO activity increased in all groups. Specific ^{3}H-imipramine binding sites showed an increase after 3 yr of heroin abuse and in all heroin plus cocaine addicts. In conclusion, short-term use of heroin decreases NE or Epi release, but with prolonged use, a slow adaptation occurs. In contrast, cocaine inhibits the neuronal Epi uptake, even in a situation of long duration of abuse. Probably the amine levels additionally regulate the amine carrier, resulting in changes that show a different pattern from major depression. These drugs of abuse may also influence directly or indirectly related enzymatic systems.

Index Entries: Heroin; cocaine; catecholamine plasma levels; monoamine oxidase B; ^{3}H-imipramine binding; addiction.

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Introduction

A great deal of research has been devoted to clarifying the biological mechanisms of drug abuse, since it was established that drug abuse is accepted as rewarding for humans and animals (Deneau et al., 1969; Di Chiara and Imperato, 1988). Among central nervous system neurotransmitters and neuromodulators, dopamine (DA) is thought to be an important component of the rewarding properties of indirect sympathomimetics, such as cocaine and amphetamine. There is also evidence that this contributes to the reinforcing properties of opiates and ethanol (Koob, 1992). Considerable evidence implicates the mesolimbic DA pathway in the rewarding effects of morphine. Systemically administered morphine-like opiates indirectly excite DA-containing neurons of the ventral tegmental area. They directly excite cells of the arcuate nucleus (Loose et al., 1990) or other catecholamine-containing neurons of the brain (Di Chiara and North, 1992). Cocaine shows DA reuptake blocking properties (Garey and Heath, 1976; Vickroy and Johnson, 1982; Smith et al., 1987) and exerts inconsistent effects on the release of DA in vivo.

Direct evidence in the human being that drugs of abuse indeed stimulate dopamine transmission in vivo is lacking as a result of the difficulties inherent in the in vivo quantitation of dopaminergic transmission. Exception is made for studies with amphetamine (Zetterstrom et al., 1983; Imperato and Di Chiara, 1984) . Since drugs of abuse interfere with the catecholaminergic activity in the periphery, studying their effects in vivo at this level could improve the understanding of central mechanism of action and explain some of the peripheral effects.

Most of the studies that have been published associate affective disorders with alterations in ^{3}H-imipramine binding. For example, a decrease in the number of binding sites has been reported in depression (Briley et al., 1980) and idiopathic chronic pains (Magni et al., 1987), whereas an increase was demonstrated after long-term treatment with MAO inhibitors (Zsilla et al., 1983) and with age (in rats) (Brunello et al., 1985; Wilson and Roy, 1985). The majority of these studies were performed in platelets, since these cells are easy to collect and have similar characteristics to serotonergic neurons (Stahl and Meltzer, 1977). It is possible that patients abusing drugs may be predisposed to depression as a consequence of the alterations in the ^{3}H-imipramine binding sites.

In the present study, central and peripheral sympathetic actions were investigated, as well as the specific ^{3}H-imipramine binding to platelet membranes. The investigation was done concerning the catecholamine and its metabolite plasma levels and activity of the MAO type B.

Methods

Subjects

All 48 participants (43 males and 5 females) were adults, ages 18–32 yr (mean ± SD: 25.45 ± 0.93 yr; median: 26 yr). Another 19 subjects (17 males and 2 females) matched by age (23.16 ± 0.38 yr; median: 23 yr) and with no history of drug abuse (referred by themselves in the interview and medical examination) and without neurologic or psychiatric disease (verified by clinical examination) were recruited by word-of-mouth referrals to be used as the control group. Before acceptance, all subjects were interviewed to explain the nature of the study and to ascertain their medical, psychiatric, and drug histories.

Thirty-eight of the 48 drug abusers (79%) currently used iv or smoked heroin (on at least one occasion in the last week), whereas 10 subjects (21%) reported simultaneous use of smoked cocaine during the last 2 yr. This last group had used heroin for more than 6 yr. Heroin abusers were grouped according to the duration of drug consumption: 1–3, 4–6, and more than 6 yr. The frequency and/or amount of drug used was not possible to measure exactly. Subjects were compelled to sign a consent form that outlined the study in detail, before participation.

Procedure

After 30 min in supine rest, blood samples (25 mL) were taken by puncture of the antecubital vein from either control subjects or drug abusers: 10 + 5 mL were collected in standard heparinized tubes for measurements of MAO activity and plasma catecholamines, respectively (to the tube for catecholamines 1 m*M* pargyline was added); another 10 mL were collected in tubes with 70 m*M* Na_2EDTA as anticoagulant for ^{3}H-imipramine binding studies. Thereafter, the tubes were placed on ice until the following procedures were performed.

Plasma Norepinephrine (NE) Epinephrine (Epi), DA, and 3-Methoxy-4-Hydroxyphenylglycol (MHPG)

These were assayed by high-performance liquid chromatography with electrochemical detection (Buchholz and Duckles, 1992). The blood samples were immediately centrifuged at 900*g* for 10 min, at 4°C, to avoid amine uptake by the erythrocytes. The plasma catecholamines were concentrated on acid-washed alumina.

Platelet Monoamine Oxidase Activity (MAO B)

The platelets were prepared using a modification of the method described by Fowler et al. (1979). The blood was centrifuged at 200*g* for 10 min to obtain platelet rich plasma (PRP). Then, the PRP was centrifuged again at 900*g*, for 15 min, and the pellet (platelets) was washed twice with an isotonic buffer (0.15 mol/L NaCl, pH 7.4) and further centrifuged at 3000*g* for 15 min. After washing, platelets were resuspended in 1.0 mL of 0.01 mol/L potassium phosphate buffer (pH 7.4) and stored at –80°C until the experiments were performed. Before use, the suspension was sonicated in a Tissumizer Desintegrator at low power for 2 min, to produce a more homogeneous preparation.

MAO activity was determined using ^{14}C-B-phenylethylamine·HCl (^{14}C-PEA, 50 mCi/mmol, New England Nuclear, Boston, MA) as a preferential substrate for MAO type B. The reaction mixture, containing 50 µL platelet suspension and 50 µL 0.01 phosphate buffer (pH 7.4) with the substrate, was incubated for 20 min; the deaminated product was extracted and measured by liquid scintillation counting (Caramona et al., 1990). The information about the K_m and V_{max} in the human platelets was obtained with PEA concentrations of 2, 4, 8, 16, 32, and 64 µ*M*. Individual measurements were performed with 100 µ*M* PEA. The results of MAO activity were expressed in nmol metabolized substrate/mg protein/h of incubation.

^{3}H-Imipramine Binding Assay

For this assay, blood was collected from control subjects (n = 19), heroin (n = 38), or heroin plus cocaine users (n = 10). The blood was sampled between 11:00 AM and 1:00 PM from the antecubital vein by dripping into polypropylene test tubes containing Na_2EDTA solution. The platelet membranes were obtained by the method of Mellerup et al. (1983) and stored at –80°C before analysis. When saturation curves were performed, the membranes from 7–10 subjects were pooled to obtain enough protein. Binding assay incubation mixtures consisted of 0.6 mL platelet membrane preparations (0.6 mg/mL protein), 0.6 mL buffer solution (with or without 10 µ*M* fluoxetine) and 0.6 mL ^{3}H-imipramine solution (0.25–8.0 n*M* for saturation curves and 2 n*M* for individual assays). Membranes and drugs were dissolved in buffer solution: 50 m*M* Tris-HCl, pH 7.4. The tubes were incubated 60 min at 1°C and then filtered under vacuum through Whatman GF/B glass fiber filters. The radioactivity bound to the filters was measured by liquid scintillation spectrometry (Packard Tricarb 460 CD). Protein concentration was determined by the method of Lowry et al. (1951) and all the assay mixtures contained the same protein concentration. Nonspecific ^{3}H-imipramine binding was obtained in the presence of 10 µ*M* fluoxetine.

The total number of binding sites (B_{max}) and the binding affinity of ^{3}H-imipramine, given by the equilibrium dissociation constant (K_d)

Table 1
Plasma Catecholamine (pg/mL) and 3-Methoxy-4-Hydroxyphenylglycol (MHPG) Contents (ng/mL) Found in Heroin or Heroin Plus Cocaine Users

Groups	*n*	NE	Epi	MHPG	DA
Control	19	516.8 ± 47.9	251.1 ± 25.1	19.7 ± 3.0	0.0
Heroin					
1–3 yr	11	292.4 ± 44.1[a]	169.7 ± 26.1[a]	23.2 ± 5.2	2.4 ± 1.5[a]
4–6 yr	11	383.4 ± 33.4[a]	174.1 ± 28.2[a]	24.4 ± 4.7	11.5 ± 6.1[a]
> 6 yr	16	441.7± 53.7	198.8 ± 31.6	26.9 ± 4.9	20.2 ± 9.9[a]
Heroin + cocaine					
	10	411.2 ± 40.6	655.7 ± 169.8[a,b]	43.3 ± 14.3[a]	27.3 ± 10.3[a]

NE: norepinephrine; Epi: epinephrine; DA: dopamine.
Results shown represent mean ± SEM.
[a]$P < 0.05$ related to control.
[b]$P < 0.05$ related to heroin consumers.

were determined using the program developed by J. Chou and T.-Chao Chou (Elsevier-Biosoft, 1985).

Statistical Analysis

Results are expressed as mean ± SEM. Student's *t*-tests were applied to evaluate statistical significance. The mean values obtained for each group of heroin users were compared to that of the control group. Heroin plus cocaine group was also compared to the group that used heroin for >6 yr. A probability of 0.05 or less was considered significant; "*n*" refers to the number of subjects of each group who participated in the study.

Results

Catecholamine Plasma Levels (Table 1)

Norepinephrine plasma levels were significantly lower in the short-term (1–3 yr, $n = 11$) and in the middle-term (4–6 yr, $n = 11$) heroin user groups compared to control subjects ($n = 19$), long-term heroin users (for more than 6 yr, $n = 16$), and heroin plus cocaine users ($n = 10$).

Epinephrine levels changed in the same way as with norepinephrine (Table 1), except that they increased still more in heroin plus cocaine abusers, and with respect to heroin users, whatever the duration of use. Conversely, MHPG plasma levels did not significantly increase with the duration of heroin use, increasing significantly only with cocaine abuse (Table 1).

DA was detected in plasma of the drug abusers group, especially when they took heroin for more than 6 yr, with or without cocaine (Table 1). In the control group, measurable DA plasma levels were not found.

MAO Activity (Table 2)

The rates of deamination in platelets sampled from controls, heroin, and heroin plus cocaine subjects were studied in order to select the concentration of ^{14}C-PEA in the experiments. The mean values of the K_m obtained were 36.6 μ*M* for platelets from controls and 39.2 μ*M* for the platelets from heroin users. Hence the concentration of PEA selected was 100 μ*M*, i.e., three times the respective K_m.

The results are summarized in Table 2. Platelet MAO activity in 19 control subjects (males and females) was not significantly different from that found in the group that used heroin for 1–3 yr. With increased time of addiction, the MAO activity became significant. The mean value of the group consuming heroin for more

Table 2
Monoamine Oxidase (MAO) Activity (nmol/mg protein/h) in Human Platelets from Control, Heroin, and Heroin Plus Cocaine Subjects

Control	Heroin groups	Heroin plus cocaine group
6.7 ± 1.0 (n = 19)	1–3 yr (n = 11): 7.6 ± 0.8 4–6 yr (n = 11): 20.1 ± 2.6[a] >6 yr (n = 16): 24.0 ± 2.9[a]	49.8 ± 6.2[a,b] (n = 10)

Results shown represent mean ± SEM.
[a]$P < 0.05$ related to control.
[b]$P < 0.05$ related to heroin consumers.

than 6 yr is about four times higher than the corresponding value for the controls. MAO activity in platelets of heroin plus cocaine abusers was significantly increased when compared to platelets from controls, even when compared to long-term heroin abusers.

^{3}H-Imipramine Binding (Tables 3 and 4)

A total of 67 subjects were included in this study. Neither male nor female heroin or heroin plus cocaine users were significantly older than controls.

Platelet membranes from each group were pooled, and used to perform saturation curves and Scatchard analyses. The K_d value for ^{3}H-imipramine affinity decreased in the heroin plus cocaine users. The maximal number of binding sites (B_{max}) increased with the length of use, especially in the heroin plus cocaine group.

When a single concentration of ^{3}H-imipramine was used (2 n*M*) for each subject, the results shown in Table 4 were obtained: The group that used heroin for 1–3 yr expressed a number of specific ^{3}H-imipramine binding sites not significantly different from controls. However, in the other heroin user groups (4–6 yr and more than 6 yr) the number of specific ^{3}H-imipramine binding sites was significantly higher. The number of specific ^{3}H-imipramine binding sites in platelets from subjects abusing heroin and cocaine was not significantly different from the heroin group with more than 6 yr of use. Nevertheless, they were significantly higher (+94%) than the control group.

Discussion

The present results suggest that heroin and cocaine, drugs belonging to different pharmacological classes, but sharing the characteristic of being rewarding in animals and humans, interfere with the catecholaminergic system. The circulating NE levels generally reflect sympathetic neural activity (Goldstein et al., 1983) since most of the NE in plasma arises from peripheral nerve endings rather than from the adrenal gland, and a positive correlation is usually assumed between sympathetic activity and plasma norepinephrine levels (Lake et al., 1976).

However, there are important limitations in the use of plasma NE as an index of sympathetic nervous activity (Shimizu and McGrath, 1993). Plasma NE is influenced not only by the rate of spillover to plasma after release, but also by the rate of clearance from plasma, which includes neuronal uptake and metabolism in nonneuronal tissue. Other confusing influences on plasma catecholamine level evaluation include collection techniques and reduced cutaneous blood flow in such conditions as cardiac failure (Shimizu and McGrath, 1993). Moreover, several mechanisms modulate the relationship between sympathetic nerve impulses and the appearance rate of NE in bloodstream. Thus, it has been proposed that opioids may act on presynaptic opioid receptors to suppress catecholamine release (Bouloux et al., 1986). Despite these objections,

Table 3
Characteristics of Specific ^{3}H-Imipramine Binding (Defined by 10 μ*M* Fluoxetine) to Platelet Membranes from Heroin or Heroin Plus Cocaine Users, Compared to Control Subjects[a]

	K_D, n*M*	B_{max}, fmol/mg protein
Control (*n* = 19)	1.96	945
Heroin		
1–3 yr (*n* = 11)	1.60	920
4–6 yr (*n* = 11)	1.21	1363
>6 yr (*n* = 16)	1.42	1780
Heroin plus cocaine (*n* = 10)	7.09	2147

[a]These values were obtained by Scatchard analysis of saturation curves performed with ^{3}H-imipramine (*see* Methods). Each experiment was always performed in triplicate.

Table 4
Characteristics of Specific 2.0-n*M* ^{3}H-Imipramine Binding (Defined by 10 μ*M* Fluoxetine) to Platelet Membranes from Heroin or Heroin Plus Cocaine Users Compared to Control Subjects[a]

Control group	Heroin groups	Heroin plus cocaine group
320.0 ± 36.8 (*n* = 19)	1–3 yr (*n* = 11): 351.5 ± 40.8 4–6 yr (*n* = 11): 432.0 ± 21.3[b] > 6 yr (*n* = 16): 640.0 ± 68.8[b]	621.6 ± 37.0[b] (*n* = 10)

[a]Each experiment was always performed in triplicate.
The values are expressed in fmol/mg protein.
Results shown represent mean ± SEM.
[b]$P < 0.05$ related to control.

venous NE provides a reasonable indirect index of sympathetic neural activity and can be used to evaluate average sympathetic tone in disease states in humans (Hjemdahl, 1993).

A significant decrease in venous plasma NE and Epi concentration without change in MHPG levels was found in this study with subjects with short- and middle-term heroin use (<6 yr of drug abuse) compared with control subjects. The mechanisms of the decreased sympathetic nerve activity in heroin users have not yet been clearly defined. However, both opioid receptors and opioid peptides are present within adrenal chromaffin tissue (Chavkin et al., 1979; Castanas et al., 1983) and there is evidence that opiates exert inhibitory modulation on catecholamine release following cholinergic nicotinic transmission at the adrenomedullary level (Kumakara et al., 1980; Costa et al., 1983). Blockade of this feedback inhibition by naloxone, a specific opiate antagonist devoid of agonist activity, could therefore lead to catecholamine release from a pheochromocytoma (Mannelli et al., 1983; Bouloux et al., 1986). In addition, it was demonstrated in experimental animals that enkephalins are able to inhibit NE release from sympathetic nerves (Ganten et al., 1984). In some nerves, it has been demonstrated that opioid peptides are costored and coreleased with NE (Linton-Dahlof and Dahlof, 1993). Although at present no strong evidence exists that opioid receptors on sympathetic noradrenergic terminals act as prejunctional inhibitory autoreceptors under physiologic conditions (Linton-Dahlof and Dahlof, 1993),

in our work, heroin significantly decreased the catecholamine spillover for years.

After long-term heroin use (>6 yr) adaptation changes could be developed, since NE and Epi levels did not significantly differ from controls. In humans, free MHPG appears to be the major brain NE metabolite released into the blood, providing a direct measure of brain NE metabolism (Demet and Halaris, 1979). Plasma MHPG levels are a less sensitive index of rapid changes in sympathetic activity than plasma NE, because they have a slower removal rate (longer half-life) than catecholamines (Kopin, 1985). Our results did not show any significant change in the MHPG plasma levels, unlike the decrease of catecholamine plasma levels. This discrepancy may signify that actually brain MHPG (and brain NE) is not decreased.

Another interesting finding in our work was the increasing DA plasma levels as the duration of heroin abuse increases, in spite of the low or normal NE and Epi concentration. A plausible explanation would be that the sympathetic system overreacts in response to the opioid-induced inhibition, increasing DA synthesis. This hypothesis must be clarified in future work.

On the other hand, our data indicate that long-term heroin use is characterized by a significant increase in the MAO type B activity. Since this increase parallels the observed increment in plasma DA concentration, there is the possibility that both phenomena are interconnected.

Finally, we have characterized the platelet serotonin carrier by using ^{3}H-imipramine, since previous studies demonstrated that in depression (Briley et al., 1980) and in chronic pain (Magni et al., 1987) its specific binding to platelet membranes was decreased. Nevertheless, and to our surprise, we found that the number of specific binding sites was significantly higher in heroin or heroin plus cocaine users, principally after long-term use. We do not know about similar results in such groups of patients. An increased number of ^{3}H-imipramine recognition sites was found after treatment of rats with (–)deprenyl for 3 wk (Zsilla et al., 1983) and in aged rats (Brunello et al., 1985; Wilson and Roy, 1985). However, our subjects did not take any inhibitor of MAO and were not old. This is a finding that is worthy of further investigation, since it is known that serotonin uptake inhibitors reduce the consumption of addictive drugs (Tyers and Hayes, 1992).

Finally, some of the differences we found between the drug users and controls could reflect differences in nutrition or some other life style difference between the groups.

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