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On the Role of Ascending Dopamine Systems in the Control of Voluntary Ethanol Intake and Ethanol Intoxication

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KIIANMAA, K , K ANDERSSON AND K FUXE *On the role of ascending dopamine systems in the control of voluntary ethanol intake and ethanol intoxication* PHARMAC BIOCHEM BEHAV **10**(4) 603–608, 1979 —Selective lesions of the ascending dopamine pathways were made by bilateral injection of the neurotoxin 6-OHDA (8 μg/4 μl) Catecholamine fluorescence histochemistry revealed a marked degeneration of the ascending mesostriatal and mesolimbic dopamine systems, while the hypothalamic dopamine and noradrenaline nerve terminals were unaffected After recovery of feeding and drinking behaviors the voluntary ethanol intake was not different from that of the controls The time of ethanol-induced narcosis and the extent of ethanol-induced hypothermia were not affected In contrast, in a tilting-plane test conducted two months after the operation, ethanol impaired the performance of the 6-OHDA-treated rats significantly less than that of the controls This finding suggests a role for the ascending dopamine neurons to the forebrain in the intoxicating effect of ethanol

Dopamine Ethanol Intoxication Catecholamine fluorescence histochemistry

BIOCHEMICAL studies indicate that ethanol can increase the turnover of brain dopamine (DA) and noradrenaline (NA) [10, 11, 13, 14, 24, 26, 36] Furthermore, pharmacological studies of the action of ethanol on locomotion and sleeping time also suggest an involvement of central catecholamines (CA) in its actions on the brain [12, 18, 19]

In addition an involvement of central CA neurons in the control of voluntary ethanol intake has been demonstrated in studies showing that marked depletion of brain NA levels by dopamine-β-hydroxylase inhibitors [5,7] or by intraventricular injections of the neurotoxin 6-hydroxydopamine (6-OHDA) cause a reduction in alcohol intake [6, 9, 30, 34, 38] However, partial depletion of the NA stores results in a transient increase in alcohol consumption [6,27]. Furthermore, the DA concentrations are higher in the brains of rats belonging to the alcohol-preferring AA strain than in rats belonging to the water-preferring ANA strain [2]

In order to further clarify the role of central DA systems in ethanol intake and in the actions of acutely administered ethanol two experiments were conducted The first experiment examined voluntary ethanol consumption and the second studied the functional effects of an acute dose of ethanol in rats following bilateral lesion of the ascending DA pathways [4,40] with the neurotoxin 6-OHDA

METHOD

EXPERIMENT 1

Three-month-old male Long Evans hooded rats were used. They were individually housed in a room having a 12-hr light-dark cycle (light period from 6 a m to 6 p m), a temperature between 22–24°C, and a relative humidity of 55% The rats had free access to Astra Evos (Sodertalje, Sweden) diet During the first 7 days they had a 10% (v/v) ethanol solution as the sole drinking fluid For the remainder of the experiment, they were continuously given a choice between tap water and the 10% ethanol solution in two 100 ml drinking tubes fitted to the front wall of the cage Intake of water and alcohol was measured daily, while the food consumption and the body weight were measured weekly

When the rats had been in a free choice situation for 3 weeks, a period long enough for the rats to establish a stable base line of alcohol consumption [21], they were divided into 2 groups matched according to their alcohol intake level

Operation Procedure

The rats were placed in a David Kopf stereotaxic instrument and were anaesthetized with a mixture of air and

halothane 6-OHDA-HCl (8 μg/4 μl, calculated as base, Labkemi, Sweden), dissolved in 0.9% saline containing ascorbic acid (0 2 mg/ml), was injected bilaterally close to the ascending DA pathways at the level of the posterior hypothalamus Fifteen minutes earlier the rats had received protriptyline (10 mg/kg, IP, Merck, Sharp and Dohme), an NA uptake blocking agent This type of procedure has been reported to cause a relatively specific degeneration of DA containing neurons [22]. The controls received the vehicle only The coordinates, according to the stereotaxic atlas of Konig and Klippel [28], were· A +3 4, L ±1 3, V −3.2

All the 6-OHDA-treated rats developed akinesia, aphagia and adipsia and had to be supported by tube-feeding During the intubation period the body weights were maintained at the preoperative level and were not different from those of the controls Those rats whose eating and drinking behaviors did not recover within 5 weeks after the operation were discarded Measurements of the alcohol intake of the remaining rats were made during the next 5 weeks

Quantitative Microfluorimetrical Measurements of CA Fluorescence

The rats were sacrificed by decapitation 3 months after the operation. The extent of the degeneration of the DA nerve terminals was evaluated by means of a quantitative microfluorimetrical analysis of the DA fluorescence in the diffuse and dotted types of DA nerve-terminals in the nucleus caudatus, nucleus accumbens and tuberculum olfactorium, using a Leitz fluorescence microspectrograph with an MPV-system The details concerning the instrumentation, the recording procedure and the anatomical subdivisions of the DA systems have been described previously [17, 29, 35].

The figures in the tables represent the specific DA fluorescence (total fluorescence minus unspecific background fluorescence). A Mann-Whitney U test was used in the statistical analysis

EXPERIMENT 2

The rats in this experiment were of the same strain and age as in Experiment 1 They were treated and operated upon in a similar manner except that they were not given access to ethanol

Ethanol Intoxication

Intoxication was measured in these rats with a tilting-plane test [8], conducted on a blind basis 2 months after the operation. In this test the animal is placed on a wire-cloth covered plane, which is tilted by a motor at a constant speed from horizontal to vertical in 5 sec The sliding angle of the rat is recorded The rats were given a pre-ethanol test and then injected with 2 g/kg of ethanol IP (12% w/v ethanol in 0.9% saline) Subsequent tilting-plane tests were performed 6 times at 20 min intervals after the injection The results after ethanol treatment were expressed as the percent of the pre-ethanol performance value for each individual, using the sine of the sliding angle as the measure of the performance. One hundred sixty minutes after the ethanol injection a blood sample of 0.05 ml was taken from the tip of the tail of each rat for gas chromatographic determination of the blood ethanol concentration [20]

A week later the effect of apomorphine on ethanol intoxication was studied in these rats using a cross-over design The rats received an injection of apomorphine (0 05 mg/kg, SC) simultaneously with the ethanol injection (2 g/kg, IP) The controls received an equal volume of saline The tilting plane test was performed three times at 20 min intervals after the injections

Ethanol-Induced Narcosis

Ethanol narcosis was induced by an injection of 4 g/kg of ethanol IP (15% w/v ethanol in 0.9% saline) The duration of the narcosis ("sleeping time") was defined as the time from the loss of the righting reflex to the time at which a rat regained this reflex. A rat was judged to have regained its righting reflex if it was repeatedly able to right itself when placed on its back A blood sample of 0.05 ml was taken from the tip of the tail of each rat at the moment of reflex recovery for gas chromatographic determination of the blood ethanol concentration

Ethanol-Induced Hypothermia

Ethanol-induced hypothermia was also estimated in the experiment on ethanol-induced narcosis Body temperature was measured by inserting a probe from an electric thermometer 4 cm into the rectum The measurements were made at 20 min intervals for 6 hours The results were expressed as the maximal temperature change relative to the pre-treatment value

Quantitative Microfluorimetrical Measurements of CA Fluorescence

The extent of degeneration of dopamine nerve terminals in the forebrain was evaluated in the same manner as described in Experiment 1 Furthermore, the CA fluorescence in various hypothalamic DA and NA terminal systems was measured as described above The DA cell bodies and the extent of the lesions in the midbrain were semiquantitatively evaluated

RESULTS

EXPERIMENT 1

The effects of the ascending dopamine pathways lesions on absolute ethanol intake are shown in Fig 1 No differences in alcohol consumption were found between the controls and lesioned animals which had recovered from adipsia and aphagia Both consumed increasing amounts of alcohol during the final 5 postoperative weeks.

As shown in Table 1, the degeneration of ascending DA pathways was considerable, particularly in the nucleus caudatus DA fluorescence in the nucleus caudatus in the 6-OHDA-treated rats was only 13.4% of the control group mean value, while the diffuse type of DA fluorescence was reduced to 17 5 and 25 0% of the control mean value in the nucleus accumbens and tuberculum olfactorium, respectively The disappearance of the dotted type of DA fluorescence, located in the posterior parts [23], was clearly less marked (37 4–42 5% of the control mean value) than that of the diffuse DA fluorescence

EXPERIMENT 2

Also in this test, all the 6-OHDA-injected rats developed akinesia, adipsia and aphagia, and were supported by tube-feeding A test on catalepsia was performed 2 months after the operation by placing the rat's front paws on a 65 mm high

TABLE 1

SPECIFIC DA FLUORESCENCE IN VARIOUS FOREBRAIN REGIONS OF RATS INJECTED WITH 6-OHDA INTO THE ASCENDING DA PATHWAYS IN EXPERIMENT 1

Group	Nucleus caudatus	Nucleus accumbens "diffuse"	Nucleus accumbens "dotted"	Tuberculum olfactorium "diffuse"	Tuberculum olfactorium "dotted"
Control	395 ± 8 (12)	320 ± 16 (10)	619 ± 29 (12)	304 ± 52 (10)	594 ± 32 (11)
6-OHDA	53 ± 15* (7)	56 ± 13* (6)	263 ± 71* (6)	76 ± 19* (6)	222 ± 56* (6)
% of control	13 4	17 5	42 5	25 0	37 4

The values are expressed in arbitrary fluorescence units (means ± SEM, n) For explanation of dotted and diffuse types of DA fluorescence see refs [23,35]

*$p<0$ 001 compared to control, Mann-Whitney U test, one-tailed

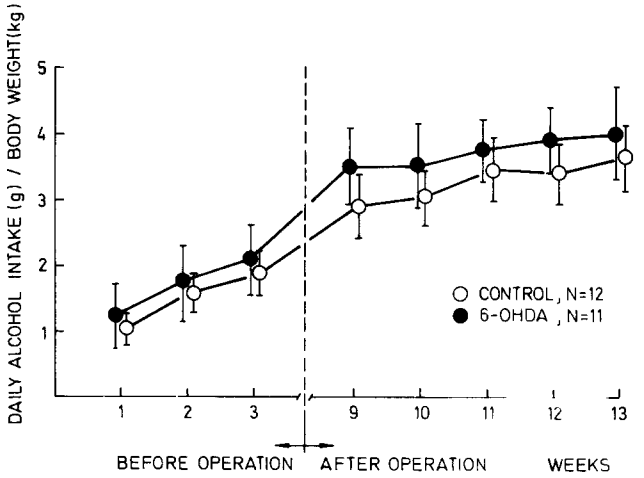

FIG 1 Effect of a 6-OHDA-induced lesion of the ascending DA pathways on voluntary ethanol intake Each point represents the mean daily absolute alcohol intake each week in g/kg body weight Means ± SEM are given

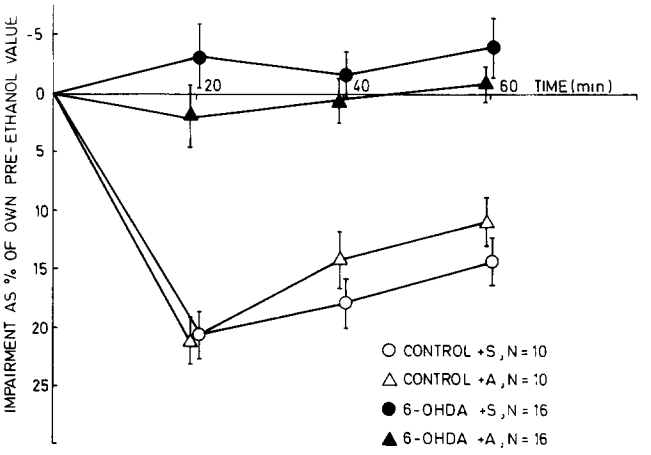

FIG 3 Effect of apomorphine on ethanol intoxication as evaluated in the tilting-plane test in control rats and in rats that had received injections of 6-OHDA close to the ascending DA pathways Apomorphine (A, 0 05 mg/kg, SC) or an equal volume of 0 9% saline (S) was injected simultaneously with a dose of 2 g/kg IP of ethanol The results are expressed as in Fig 2

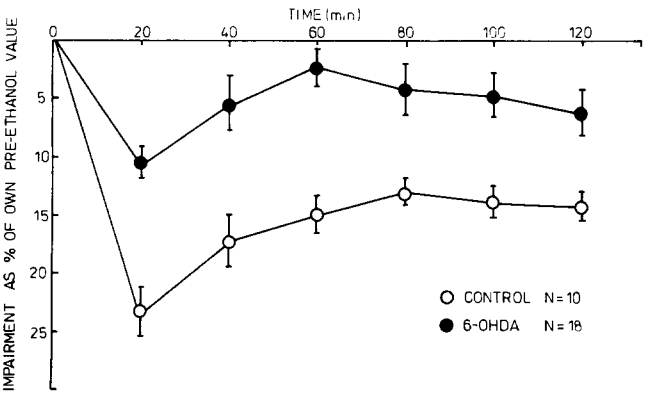

FIG 2 Effect of a 6-OHDA-induced lesion of the ascending DA pathways on ethanol intoxication in the rat as evaluated in the tilting-plane test Ethanol was administered in a dose of 2 g/kg IP Each point represents the mean impairment of performance in percent of the value (= sine of the sliding angle) obtained before ethanol treatment Means ± SEM are shown A profile analysis [33] revealed a highly significant difference in the level of performance between the groups ($t=5$ 513, $df=26$, $p<0$ 001)

block using a cutoff time of 300 sec. The 6-OHDA-treated animals stayed in this position for 122 ± 24 sec (mean ± SEM), while the controls stayed only 5 ± 2 sec ($t=3$ 810, $df=24$, $p<0$ 001).

Ethanol Intoxication

The effects of the lesions on ethanol intoxication are shown in Fig 2. The ethanol-induced impairment of performance in the tilting-plane test in the 6-OHDA-treated group was significantly less than in the controls throughout the experiment There was no difference in the performances of the 6-OHDA-treated group (59 ± 1 degrees; mean ± SEM) and of the control group (58 ± 2 degrees) in this test before ethanol treatment The blood ethanol concentrations of the 6-OHDA-injected rats (38.5 ± 2 6 mM; mean ± SEM) were not significantly different from those of the controls (34 6 ± 0.9 mM).

The effects of apomorphine on the tilting-plane performance in both the control and 6-OHDA-treated rats are shown in Fig. 3 In agreement with the results from the first test (shown in Fig 2) the 6-OHDA-treated animals showed less impairment of performance from ethanol than did the con-

TABLE 2

ETHANOL-INDUCED NARCOSIS AND HYPOTHERMIA IN DA LESIONED AND CONTROL ANIMALS

Parameters	Control group	6-OHDA group
Narcosis time (min)	184 ± 15 (12)	217 ± 15 (18)
Blood ethanol concentration (mM)	88 3 ± 1 6 (12)	85 7 ± 1 3 (18)
Maximal hypothermia (°C)	2 9 ± 0 2 (12)	3 1 ± 0 1 (16)

Means ± SEM and n are given For details see material and methods section

TABLE 3

SPECIFIC DA FLUORESCENCE IN VARIOUS FOREBRAIN REGIONS OF RATS INJECTED WITH 6-OHDA INTO THE ASCENDING DA PATHWAYS IN EXPERIMENT 2

Group	Nucleus caudatus		Nucleus accumbens		Tuberculum olfactorium	
	"diffuse"	"dotted"	"diffuse"	"dotted"	"diffuse"	"dotted"
Control	214 ± 14 (9)	308 ± 15 (9)	162 ± 11 (8)	395 ± 31 (9)	168 ± 16 (8)	307 ± 24 (8)
6-OHDA	7 ± 2* (12)	13 ± 1* (13)	14 ± 2* (13)	38 ± 17* (13)	27 ± 4* (12)	37 ± 11* (13)
% of control	3 3	4 2	8 6	9 6	16 1	12 1

The values are expressed in arbitrary fluorescence units (mean ± SEM, n) For explanation of dotted and diffuse types of DA fluorescence see refs [23, 35]

*$p<0.001$ compared to control, Mann-Whitney U test, one-tailed

TABLE 4

SPECIFIC CA FLUORESCENCE IN VARIOUS HYPOTHALAMIC REGIONS OF RATS INJECTED WITH 6-OHDA INTO THE ASCENDING DA PATHWAYS IN EXPERIMENT 2

Group	SEL	MPZ	LPZ	PV II	DM	PV I	PA FP	PA FM
Control	33 ± 2 (9)	65 ± 4 (9)	85 ± 2 (9)	23 ± 1 (8)	41 ± 2 (8)	27 ± 1 (8)	45 ± 2 (8)	51 ± 2 (8)
6-OHDA	34 ± 1 (14)	59 ± 3 (14)	83 ± 2 (14)	22 ± 1(14)	43 ± 2 (14)	29 ± 1 (15)	49 ± 1 (15)	55 ± 2 (15)
% of control	103	91	98	96	105	107	109	108

The fluorescence values are expressed in arbitrary fluorescence units (means ± SEM, n)

In the statistical analysis a Mann-Whitney U test was used SEL=subependymal layer of the median eminence (ME), MPZ=medial palisade zone of the ME, LPZ=lateral palisade zone of the ME, PV II=posterior periventricular hypothalamic region, DM=dorsomedial hypothalamic nucleus, PV I=anterior periventricular hypothalamic region, PA FP=parvocellular paraventricular hypothalamic nucleus, PA FM=magnocellular paraventricular hypothalamic nucleus

trols, in fact, the lesioned animals showed no impairment Apomorphine in the dose used had no effect on performance after ethanol administration in the vehicle injected controls or in the 6-OHDA group

Ethanol-Induced Narcosis and Hypothermia

As shown in Table 2, the duration of the ethanol-induced narcosis was not significantly increased by ethanol The blood ethanol concentrations at the time of righting reflex recovery were not significantly different Also the maximal decrease in body temperature was similar in the two groups

Quantitative Microfluorimetry of CA Fluorescence in the Forebrain and the Hypothalamus

The quantitative microfluorimetrical analysis of DA fluorescence in the forebrain of rats killed 4 months after operation revealed a very marked degeneration of the DA systems in all forebrain areas analyzed (Table 3) The specific DA fluorescence that remained was only 3 3 to 16 1% of the control group mean value, the effects being most pronounced in the nucleus caudatus The DA cell bodies (A8–A10, see ref [16]) in the midbrain had almost completely disappeared in the 6-OHDA-treated group, probably via a retrograde cell body degeneration Various types of hypothalamic DA and NA nerve terminals were unaffected by the 6-OHDA-induced lesions of the forebrain (Table 4)

DISCUSSION

The results of Experiment 1 show that in spite of substantial degeneration of ascending dopamine pathways that innervate the nucleus accumbens, the tuberculum olfactorium and the nucleus caudatus, no change in alcohol intake was seen 5 weeks following the operation at a time when the rats had recovered from aphagia and adipsia This finding may suggest that the ascending DA neurons are not important for the central control of alcohol intake in rats This view is supported by previous work [6,9] showing that rats receiving intraventricular injections of 6-OHDA in combination with pargyline and the NA uptake blocking agent desipramine did not reduce the ethanol intake significantly In contrast, rats having deficits in both brain noradrenaline and dopamine via combined treatment with 6-OHDA and pargyline showed a significant reduction in ethanol intake On the basis of these findings the authors concluded [6,9] that brain noradrenaline is more important than dopamine for the regulation of voluntary intake of ethanol in rats (see also Ref [30]), which is also supported by our earlier findings that partial depletion of forebrain noradrenaline causes an increase in ethanol intake [27] Finally, on the basis of Experiment 2, it is unlikely that the hypothalamic NA nerve terminals were lesioned in the present study, suggesting that the ascending DA pathways were selectively lesioned

A role for the DA neurons in ethanol intake cannot, however, be excluded The fact that the rats were no longer adipsic or aphagic shows that functional recovery must have occurred It has been suggested that the surviving CA neurons are able to compensate for the 6-OHDA-induced losses of CA nerve cells [39] A compensatory enhancement of dopamine turnover occurs in the remaining dopamine neurons [1] and receptor supersensitivity develops [32,41] The transfer of function of the damaged pathways to other types of nerve cells with another transmitter is also a possible compensatory mechanism [39] Perhaps these or other compensatory processes in the brain are responsible for the finding that no change in alcohol consumption was seen five weeks after the operation Thus, it is still possible that the destruction of the DA pathways led to an early effect on alcohol intake, which was missed because of the motor deficits found during this period

Although destruction of the ascending dopamine pathways did not affect ethanol consumption in Experiment 1, a similar lesion in Experiment 2 markedly inhibited ethanol intoxication in rats measured with the tilting-plane test This cannot be explained in terms of a difference in the blood ethanol concentration, since this was similar in both groups at the end of the experiment The motor impairment found in the lesioned animals is likewise an improbable explanation, because no difference was found in the performance before ethanol treatment and hence the 6-OHDA treated animals performed better after ethanol intoxication. It has been found that akinetic animals when stimulated sufficiently, as in an underwater swimming maze [37], are able to perform

A relationship between ethanol and the dopamine systems has previously been suspected. An acute administration of ethanol has been shown to activate central dopaminergic neurons [10, 13, 26] It may therefore be speculated that the dopaminergic neuron systems are involved in expression of the effects of an acute dose of ethanol and that the lesion therefore reduces the expression of intoxication In line with this interpretation is the finding that apomorphine increased the intoxicating effects of ethanol in man [3], and the fact that the 6-OHDA-treated rats maintained their akinetic state throughout the experiment, indicating no recovery of function in the ascending DA pathways

Other transmitter systems may also play a role in the expression of the actions of acute ethanol treatment in the present experiments Thus, GABA neurons may also be involved, because a gabaergic drug, aminooxyacetic acid, enhances and a GABA antagonist, bicuculline, diminishes ethanol intoxication in rats [25] Furthermore, ethanol-induced locomotor stimulation is suppressed by GABA-like drugs [15]

The present findings that the duration of ethanol-induced narcosis and the ethanol-induced hypothermia were not affected, are somewhat contradictory to previous results Prolonged ethanol-induced narcosis in mice has been reported after treatment with α-methyl-p-tyrosine [19], and a reduced duration of narcosis after *d*-amphetamine [19] or amantadine [31] treatment It therefore remains to be determined whether or not DA is involved in these effects of ethanol

In conclusion, the present results suggest that the ascending DA systems to the forebrain are not important for the central control of voluntary ethanol intake in rats A role of the ascending mesotelencephalic DA systems in the ethanol-induced impairment of motor coordination is, however, suggested based on the finding that selective degeneration of these neurons decreases the intoxicating effects of ethanol in the tilting-plane test

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