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Combined Effects of n-Hexane and Toluene on Norepinephrine and Dopamine Levels in Rat Brain Tissues after Long-Term Exposures

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Recent studies have demonstrated that catecholamines in the brain tissues play important roles as chemical neurotransmitters, and various studies have been conducted from the viewpoints of neurochemistry, neurophysiology as well as neuropharmacology (Nagatsu 1973). Reports of the effects of exposure to organic solvents on biogenic amines are however still limited (Honma et al. 1980; Honma 1983; Honma et al. 1983; Arito et al. 1984; Rea et al. 1984), although organic solvents are a well-known group of neurointoxicants. The present study was initiated to examine 1) whether long-term exposure to organic solvent vapor gives any effects on the levels of norepinephrine (NE) and dopamine (DA) in various brain regions, 2) whether the brain catecholamine levels respond specifically to the central neurotoxicity of the solvent separately from the peripheral neurotoxicity, and 3) whether there occurs any combined effects on solvent toxcity when two solvents are administered simultaneously. For these purposes, n-hexane and toluene were selected as model solvents with peripheral neurotoxicity and central neurotoxicity after long-term exposure, respectively (Sandmeyer 1981).

MATERIALS AND METHODS

Male Wistar rats (weighing ca. 230 g at the beginning of the experiments) were used. The animals were separated into 4 groups (5 animals per group) as follows: 1) The control group to be sham-exposed to fresh air. 2) The n-hexane group to be exposed to n-hexane vapor. 3) The toluene group to be exposed to toluene vapor. 4) The mixture group to be exposed to a mixture of vapors of n-hexane and toluene. Two experiments were conducted; in the first experiment, the concentrations employed were 200 ppm for n-hexane, 200 ppm for toluene, and 200 ppm (n-hexane) plus 200 ppm (toluene) for the mixture, while the levels were 400 ppm for n-hexane, 400 ppm for toluene, and 200 ppm

(n-hexane) plus 200 ppm (toluene) for the mixture in the second experiment. In each experiment, the animals were caged in groups and exposed to the vapors continuously for 30 days in a servomechanized exposure system with four exposure chambers built in parallel (Koizumi and Ikeda 1981). The mean observed concentrations were within 93-107% of the respective ordered concentrations with CV of less than 5% (n=172) as previously reported (Kumai et al. 1984). During the time period of 9:30 to 11:30 AM on the morning of the last day of the exposures, the animal was sacrificed by decapitation, and brain was isolated and dissected into 11 regions after the method of Glowinski and Iversen (1966) with a modification to separate the cerebral cortex into 3 regions (Tables 1 to 3). Each tissue was weighed and then stored at -80°C till analysis. For analysis, the tissue was homogenized in 0.4N $HClO_4$ and the deproteinized supernatant solution was obtained by centrifugation. After adjustment of the pH of the solution to 8.5, the amines in the solution were absorbed on alumina (activated after Anton and Sayre 1962) and desorbed into 0.1N HCl, and an aliquot (25 µl) of the eluate was injected into a Hitachi high performance liquid chromatograph (Model 635A) equipped with a Hitachi Gel #3056 column (4 mm in inner diameter and 150 mm in length). NE and DA were eluted with 0.04M KH_2PO_4-0.05% H_3PO_4 at a flow rate of 0.6 ml/min (back pressure; 60-80 kg/m^2) and measured at an excitation wavelength of 280 nm (slit width; 10 nm) taking advantage of natural fluorescence after Scratchley et al. (1977). The recovery of NE was 94.0± 9.2% (mean±SD, n=5) and 95.2±7.4%, respectively, when 3 ng and 10 ng of the authentic NE were applied on the activated alumina. The space of the peak in the chromatogram was linearly related to the added amount of both NE and DA, respectively. For statistical evaluation of the difference from the corresponding control, Student's t-test was employed with an assumption of normal distribution.

RESULTS AND DISCUSSION

Weight gain during the one month exposure period was significantly less in the groups exposed to either n-hexane ($p<0.05$) or toluene ($p<0.05$) at 200 or 400 ppm than in the control group. In the mixture group, the reduction in weight gain was significant only in one experiment ($p<0.05$), but insignificant ($p>0.10$) in the other experiment and also when the results of the two experiments were combined. No significant difference ($p>0.10$) was found among the 4 groups in the weight of liver or brain. No clinical sign of peripheral neuropathy was observed in any groups including those exposed to n-hexane at 200 or 400 ppm.

Table 1. NE and DA in various brain regions of rats exposed to n-hexane (200 ppm), toluene (200 ppm) or the combination (n-hexane at 200 ppm and toluene at 200 ppm)

Regions	Control	n-Hexane	Toluene	Mixture
Norepinephrine (ng/g wet tissue)				
Dorsal cortex	70± 20	101± 28	60± 15	77± 12
Ventral cortex	95± 28	81± 27	135± 64	87± 26
Olfactory cortex	242± 63	295± 89	217± 24	315± 75
Frontal cortex	161± 51	181± 85	154± 22	156± 20
Midbrain	433± 83	472± 212	392± 34	376± 71
Thalamus	313± 94	561± 276	332± 17	284± 40
Pons+Medulla	496± 165	341± 131	376± 41	429± 68
Hippocampus	265± 78	328± 106	249± 20	323± 53
Cerebellum	344± 65	378± 177	366± 58	357± 16
Striatum	155± 51	143± 13	148± 22	151± 17
Hypothalamus	1366± 267	1334± 250	1128± 334	1164± 128
Dopamine (ng/g wet tissue)				
Dorsal cortex	19± 6	17± 7	17± 3	17± 4
Ventral cortex	138± 42	108± 41	146± 25	144± 21
Olfactory cortex	1243± 188	1117± 208	1168± 373	1170± 123
Frontal cortex	139± 44	145± 29	123± 20	137± 21
Midbrain	176± 41	332± 215	152± 17	133± 17††
Thalamus	49± 20	47± 16	52± 13	46± 7
Pons+Medulla	46± 17	42± 16	41± 5	43± 7
Hippocampus	15± 2	13± 4	14± 3	21± 5***
Cerebellum	16± 3	17± 5	15± 4	14± 4
Striatum	5458±1504	4539±1623	5139±1263	5336±1464
Hypothalamus	788± 181	551± 187††	720± 147	598± 154†

Values are means±SD(n=5). Asterisks and daggers indicate that the difference from the corresponding control is statistically significant (***, **, * for increase with $p<0.01$, 0.05 and 0.01, and †††, ††, † for decrease with $p<0.01$, 0.05 and 0.10, respectively).

Table 2. NE and DA in various brain regions of rats exposed to n-hexane (400 ppm), toluene (400 ppm) or the combination (n-hexane at 200 ppm and toluene at 200 ppm)

Regions	Control	n-Hexane	Toluene	Mixture
Norepinephrine (ng/g wet tissue)				
Dorsal cortex	80± 20	131± 35**	66± 18	81± 13
Ventral cortex	98± 28	117± 29	129± 24*	84± 17
Olfactory cortex	294± 28	383± 60**	242± 21††	379± 51**
Frontal cortex	173± 30	271± 76**	194± 27	195± 35
Midbrain	366± 71	497± 154	348± 37	423± 84
Thalamus	329± 78	679± 157***	315± 25	301± 58
Pons+Medulla	430± 75	352± 76	381± 42	453± 131
Hippocampus	314± 54	314± 97	281± 19	303± 63
Cerebellum	322± 58	549± 121**	368± 44	420± 70
Striatum	190± 21	164± 29	169± 16	166± 32
Hypothalamus	1312± 225	1205± 198	1066± 125†	1263± 141
Dopamine (ng/g wet tissue)				
Dorsal cortex	21± 8	24± 4	22± 4	21± 5
Ventral cortex	146± 29	107± 20††	138± 41	144± 20
Olfactory cortex	1134± 94	1545± 398*	1559± 417*	1272± 69**
Frontal cortex	187± 27	178± 28	183± 25	177± 12
Midbrain	171± 32	201± 23	191± 20	148± 16†
Thalamus	51± 12	55± 11	64± 23	52± 5
Pons+Medulla	49± 9	51± 10	57± 7	60± 9*
Hippocampus	18± 6	17± 4	20± 4	22± 6
Cerebellum	14± 4	18± 4	17± 7	18± 3*
Striatum	4377± 151	4104± 75†††	3971± 374††	3949± 112†††
Hypothalamus	771± 79	755± 38	788± 90	618± 38†††

Values are means±SD(n=5). Asterisks and daggers indicate that the difference from the corresponding control is statistically significant (***, **, * for increase with $p<0.01$, 0.05 and 0.01, and †††, ††, † for decrease with $p<0.01$, 0.05 and 0.10, respectively).

Table 3. Percent changes in NE and DA

Regions	n-Hexane		Toluene		Mixture		
	200 ppm	400 ppm	200 ppm	400 ppm	n-Hexane 200 ppm +Toluene 200 ppm		
					I[a]	II[b]	I+II[c]
Norepinephrine							
Dors.cor.	144	164**	86	83	110	101	105
Vent.cor.	85	120	142	132*	92	86	89
Olfact.cor.	122	130**	90	82††	130	129**	129**
Front.cor.	112	157**	96	112	97	113	105
Midbrain	109	136	91	95	87	116	100
Thalamus	179	206***	106	96	91	91	91
Pons+Med.	69	82	76	89	86	105	95
Hippocampus	124	100	94	89	122	96	108
Cerebellum	110	170**	106	114	104	130	117**
Striatum	92	86	95	89	97	87	92
Hypothalamus	98	92	83	81†	85	96	91
Dopamine							
Dors.cor.	89	114	89	106	89	100	95
Vent.cor.	78	73††	106	95	104	99	101
Olfact.cor.	90	136*	94	138*	94	112**	103
Front.cor.	104	95	88	98	99	95	96
Midbrain	188	118	86	112	76††	87†	81†††
Thalamus	96	108	106	126	94	102	98
Pons+Med.	91	104	89	116	93	122*	108
Hippocampus	87	94	93	111	140***	122	130***
Cerebellum	106	129	94	121	88	129*	107
Striatum	83	94†††	94	91††	98	90†††	94
Hypothalamus	70††	98	91	102	76†	80†††	78†††

Values are means (n=5 unless otherwise specified).
Asterisks and daggers mean as in Table 1.
a Experiment 1. b Experiment 2. c Combination (n=10).

In the rats exposed to either n-hexane or toluene at 200 ppm, essentially no significant ($p>0.10$) change was observed in both NE and DA levels in any brain regions (Tables 1 and 2). The DA level in midbrain of the n-hexane group was high in some animals, resulting in apparent elevation in the group mean, but the change was statistically insignificant because SD was large (Table 1). Although the DA level in hypothalamus of the same group (i.e., exposed to n-hexane at 200 ppm) was significantly lower ($p<0.05$) than that of the control, the findings were not reproduced in the animals exposed to the same solvent

at a higher concentration of 400 ppm.

Exposures of the animals to solvent vapors at 400 ppm resulted in more marked changes especially in the NE levels (Table 2). In the group exposed to n-hexane, the NE level was significantly elevated in thalamus ($p < 0.01$) as well as dorsal, olfactory and frontal cortex, and cerebellum ($p<0.05$). Reduction of DA in ventral cortex ($p<0.05$) and striatum ($p<0.01$) was significant, while DA elevation in olfactory cortex was of borderline significance ($p<0.10$) (Table 2). Toluene exposure at 400 ppm brought about much less changes; the significant ($p<0.05$) reduction was observed only in NE in olfactory cortex and in DA in striatum, and other changes such as NE elevation in vertical cortex and reduction in hypothalamus (Table 2) and DA elevation in the olfactory cortex were only marginally significant ($p<0.10$) (Table 2).

When the animals were exposed to a mixture of n-hexane (200 ppm) and toluene (200 ppm), note-worthy changes were found in DA levels in several regions, i.e., significant decrease ($p<0.01$ for both) in midbrain and hypothalamus and significant increase ($p<0.05$) in hippocampus, which were not observed in the animals exposed to either n-hexane or toluene alone. It should also be noted that the changes were essentially reproducible in the two experiments, even though the statistical significance of the difference from the concurrent controls varied depending on the experiments (Table 3).

The results of the present study indicate that the changes of both NE and DA levels in various brain regions were not remarkable after long-term exposure of rats to either n-hexane or toluene alone at 200 ppm. Thus, the two amine levels cannot be considered as sensitive indicators of the exposure to the two solvents, bearing in mind that the exposure conditions employed (i.e., n-hexane or toluene at 200 ppm for 24 hours a day for 30 days continuously) were 6 to 15 times as intense as current occupational exposure limits for n-hexane (40 ppm) and toluene (100 ppm) (Japan Association of Industrial Health 1985). The negative findings on the sensitivity are in agreement with previous reports. For example, in a similar experiment conducted by Honma et al. (1983) in which rats were exposed for 30 days continuously to toluene at 200, 400 and 800 ppm, significant ($p<0.05$) reduction in NE in hypothalamus was observed only in the animals exposed at 400 ppm but not at 200 nor 800 ppm, while the increase of DA in striatum as well as decrease of NE in cortex plus hippocampus was statistically insignificant at all the exposure levels

employed. After a single 8-hour exposure of rats to toluene at 100, 300 and 1000 ppm, Rea et al. (1984) found no remarkable changes in NE nor in DA on whole brain basis; significant elevation was detected only in DA in striatum of the animals exposed at 1000 ppm.

At a higher exposure level of 400 ppm, changes were more remarkable in DA levels rather than in NE (Table 3). Worthy to note is the fact that the brain regions with significant changes ($p<0.05$) in NE or DA levels were more in the n-hexane group than in the toluene group. Such findings appear to be contradictory to clinical experiences that toluene is primarily a central nervous system intoxicant while the major target of n-hexane toxicity after long-term exposures is the peripheral nervous system (Sandmeyer 1981).

Exposure of the animals to a mixture of n-hexane and toluene vapors both at 200 ppm produced changes in NE and DA levels which were not predictable from the findings with the exposure to either solvent alone (Table 3). While NE elevation in olfactory cortex and cerebellum of the animals exposed to the vapor mixture was paralleled by the similar changes in the n-hexane group, the changes in DA levels such as elevation in hippocampus and reduction in midbrain and hypothalamus, especially those in the former two regions, were not associated with analogous changes in the same brain regions of the animals exposed to either n-hexane or toluene alone (Table 3). Honma (1983) also reported, even though after exposures at much higher concentrations of 1000 to 8000 ppm for single 8-hour period, that the acetylcholine level in hippocampus was lower in the rats exposed to n-hexane at 2000 to 8000 ppm in combination with toluene at 4000 ppm than in the rats exposed to toluene at 4000 ppm alone. Takeuchi et al. (1981) disclosed that the peripheral neurotoxicity of n-hexane, as measured by the reduced nerve conduction velocity in the tail of the rat exposed, is alleviated by the co-exposure to toluene. The present findings suggest the possibility that these two most popular constituents of commercial organic solvent preparations may interact also in the central nervous system probably towards the direction of toxicity potentiation, when given simultaneously.

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