Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 2, pp. 261–264, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00090-9

Tail Pinch Increases Acetylcholine Release in Rat Striatum Even After Toluene Exposure

K. STENGÅRD*†

*Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institute, Stockholm, Sweden
†Department of Neuromedicine, National Institute of Occupational Health, Solna, Sweden

Received 10 April 1994; Revised 12 May 1994; Accepted 12 May 1994

STENGÅRD, K. *Tail pinch increases acetylcholine release in rat striatum even after toluene exposure.* PHARMACOL BIOCHEM BEHAV 52(2) 261–264, 1995. – The effect of tail pinch on acetylcholine release in the striatum of freely moving rats was studied by microdialysis immediatly after inhalation exposure to toluene (2000 ppm, 2 h) or exposure to air only. It has recently been found that toluene increases extracellular dopamine levels while decreasing acetylcholine release, and that dopamine uptake inhibition increases both extracellular dopamine levels and acetylcholine release, suggesting that toluene decreases acetylcholine release by a dopamine-independent mechanism. The present experiment was an attempt to study if a behaviourally induced increase of extracellular dopamine differs from that induced by toluene in affecting striatal acetylcholine release. Acetylcholine release increased during tailpinch in the unexposed as well as the toluene exposed group. No difference between the two groups in the acetylcholine release response to tailpinch was demonstrated. The result supports the conclusion that acute toluene exposure decreases acetylcholine release via a dopamine independent mechanism, and suggests that toluene exposure does not affect the striatal acetylcholine response to an acute stressful stimulus.

Tail pinch ACh release Toluene exposure Rat striatum Microdialysis

TOLUENE is an aromatic hydrocarbon with widespread use in industry. In humans, toluene exposure causes symptoms releated to the central nervous system, such as headache, dizziness, and prolonged reaction time [for review, see (4)]. Within the rat striatum, toluene increases the extracellular dopamine (DA) level (23), downregulates the DA D_2 receptor (7,12,13), and decreases acetylcholine (ACh) release (24). In contrast, a DA uptake inhibitor, which also induces a rise in the extracellular DA levels, increases the striatal ACh release (24). These observations suggest that toluene affects striatal ACh release by a DA independent mechanism.

Tail pinch is regarded as a stressful activating stimulus (3) and induces striatal DA release in the rat (6,22). The effect of tail pinch on striatal ACh release is unknown, although restraint stress and handling increase ACh release in the hippocampus but not in the striatum (17,20).

The neuronal dependence of extracelular ACh levels monitored with in vivo microdialysis is well established (9,10,19). The present study was made in an attempt to monitor, using in vivo microdialysis (26), the effect of tail pinch on striatal ACh release in rats exposed to toluene or air (control). Thereby, information could possibly be obtained whether the increase in extracellular DA induced behaviourally (by tail pinch) differs from that induced by toluene in affecting striatal ACh release.

METHOD

Male Sprague-Dawley rats (B&K Universal AB, Sollentuna, Sweden), body weight 290–315 g, were anesthetized with halothane (1.5% in air). A microdialysis probe (membrane length 2 mm, CMA 12/CMA Microdialysis AB, Stockholm, Sweden) was stereotaxically implanted into the dorsal striatum. Coordinates in mm were anteriorly = 2.0; laterally = 2.3, and dorsally = 6.2, according to the atlas by Paxinos and Watson (21). The probe was secured to the skull using dental cement and two tiny screws. After surgery, the rats were placed in separated home cages for 48 h with free access to food and water. The Ethical Committee (Stockholms Norra Djurförsöksetiska Nämnd, Dnr N54/92) had accepted the methods used in this study prior to the start of the experiment.

On the day of the experiment the rat was placed in an

Requests for reprints should be addressed to Karl Stengård, Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institute, S-171 77 Stockholm, Sweden.

exposure chamber. The chamber, which has been described in detail before (23), was made of stainless steel and Plexiglas, and had a glass front door. The rat compartment was 30 × 30 × 30 cm. Total chamber volume was 50 liters. The chamber was airproof, and air was supplied at constant flow (10 l/min) from a small compressor. Toluene (Merck Darmstadt, Germany, art. nr 8331) was added to the air in a vaporizer (Fluotec, Cyprane Ltd, Keighley, England). The microdialysis probe tubings were connected to a two-channel liquid swivel at the roof of the rat compartment. The inlet of the swivel was connected to a microinfusion pump (CMA 100/CMA Microdialysis AB) with a syringe containing the perfusion medium. The outlet of the swivel was connected to a refrigerated microsampler, temperature 4°C (CMA 200/CMA Microdialysis AB). The microinfusion pump and the refrigerated microsampler were located outside the exposure chamber. The tubings between the swivel and the microdialysis probe did not noticeably hinder the movement of the rat. Perfusion medium was artificial cerebrospinal fluid (CSF; Apoteksbolaget, Stockholm, Sweden) containing; NaCl (147 mM), KCl (2,7 mM), $CaCl_2$ (1,2 mM), $MgCl_2$ (0.85 mM); pH 6. Neostigmine (1 μM) was added to the artificial CSF to facilitate detection of ACh (19). Perfusion speed was 2.0 μl, and perfusates were collected every 20 min.

Toluene exposure started 1 h after the tubing from the microdialysis probe had been connected to the microinfusion pump. The toluene exposure lasted 2 h. The toluene concentration was 1957 ± 238 ppm (mean ± SEM). The concentration of toluene within the animal compartment was determined every 30 min by a gas chromatograph.

Tail pinch was made by placing a paper clip on the base of the rat's tail for 5 min. The paper clip was applied 3 h after the sampling begun. Thus, tail pinch was performed immediately after termination of the toluene exposure in the toluene exposed group. In an attempt to mimic the handling of the rats when the paper clip was applied, control rats were handled briefly at the same time as the paper clip was applied on the tail pinched rats.

Acetylcholine Analysis

ACh was determined by liquid chromatography with electrochemical detection by use of immobilized enzymes (19). The samples were placed in a refrigerated autoinjector (CMA 200, CMA/Microdialysis AB) and 10 μl were injected into a HPLC system. This system has been described in detail previously (19). Briefly, ACh and choline were first separated on a polymeric column using phosphate buffer containing 1 mM sodium 1-octanesulfonate as ionpairing reagent. An enzymatic post column reactor with immobilized aceylcholinesterase and cholineoxidase transformed ACh and choline to hydrogen peroxide and betaine. Hydrogen peroxide was then electrochemically detected at a platinum electrode, which was set at 500 mV (vs. Ag/AgCl). External standards, automatically injected between every fifth sample, were used to calibrate the concentrations of ACh in the perfusate samples. The limit of detection was 0.2 pmol/10 μl. In this report, only ACh values are presented, although choline values are always detectable in the assay.

Statistics

The statistics were calculated on the percentage of pre tail pinch value. The effect of tail pinch within each group was tested by one-way analysis of variance (ANOVA) with Newman-Keuls post hoc analysis; $p < 0.05$ being used as limit for demonstration of a difference. All results are presented as mean ± SEM.

RESULTS

The pretail pinch value concentrations of ACh release in the striatum were: control group 374 ± 63 nM; tail pinch group 312 ± 46 nM, and toluene and tail pinch group 293 ± 55 nM, respectively.

The effect of tail pinch on ACh release in the striatum of rat is shown in Fig. 1. Tail pinch increased ACh release up to 20 min after the pinch in the unexposed (air only) rats (to about 140% of pretail pinch value), as well as in those exposed to toluene (2000 ppm, 2 h; to about 152% of pretail pinch value). There was no clear effect on ACh release by the handling in the control group.

The behavior of the rats was changed during tail pinch. The motor activity increased, and the rats started to lick and gnaw on the paper clip. The toluene-exposed group behaved similarly during tail pinch, but the onset was slightly delayed. The control group did not seem to be affected by the handling.

DISCUSSION

The present study showed that tail pinch increases ACh release in the rat striatum, while normal handling has no such clear effect. Toluene exposure did not demonstrably affect the increase in ACh release caused by tailpinch.

Mild tail pinch of rats induces behavioural changes, e.g., eating, gnawing, and licking, which are dependent on the nigrostriatal DA system (3). In addition, microdialysis recordings and in vivo voltammetry have demonstrated that tail pinch increases DA release within the striatum (6,22). Striatal

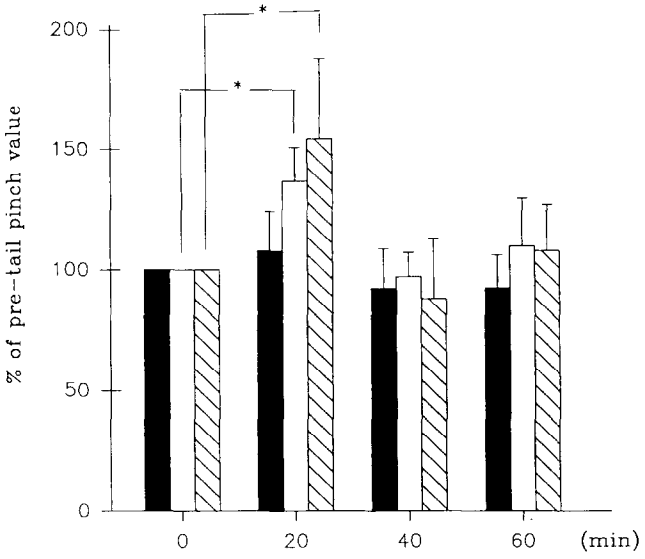

FIG. 1. Acetylcholine release in striatum of awake, freely moving rats before, during and after tail pinch, expressed as percentage of pretail pinch value. Significance of the boxes; black = control (n = 4); white = tail pinch (n = 6); hatched = toluene and tail pinch (n = 5). Tail pinch was made at time point 20 min for 5 min. Control rats were handled simultaneously with the application of pinch in the other groups. Exposure to toluene (1957 ± 238 ppm, 2 h) was made immediatly before tail pinch. Asterisk (*) indicates $p < 0.05$.

DA release is also increased by handling or restraint stress (8,17), while the latter stimulus does not increase striatal ACh release (17). Contrastingly, in the hippocampus, ACh release is increased by handling (18) as well as restraint stress (14,15,17). The increased release of striatal ACh seen in the present study is probably due to the tail pinch and not the concomitant handling of the animal, as handling alone did not demonstrably increase ACh release.

Acute toluene exposure is known to increase extracellular DA levels, while the extracellular levels of the DA metabolite HVA remains unaffected (23). The fact that the HVA levels are unaffected indicates that DA metabolism and synthesis are unaffected by acute toluene exposure. The DA synthesis is regulated by the dopamine D_2 autoreceptor (27), and blockade of this receptor causes a strong increase in extracellular HVA levels (28). In contrast, inhibition of DA uptake increases extracellular DA levels, while HVA levels remains unaffected (16). Thus, it has been suggested that interference with the DA reuptake site is a possible mechanism for the effect of toluene on DA transmission.

It is well documented that DA influences ACh release within the striatum. DA receptors, D_1 and D_2, are thought to have different effects on ACh release. D_1 stimulates ACh release, while D_2 inhibits the release (2,5,11,18,25). Tail pinch causes a sharp transient increase (240%) in the extracellular striatal DA levels the first 10 min after the pinch (6,22). The enhanced DA levels returned to baseline values within 30 min after the pinch (6,22). In the present study, increased ACh release the first 20 min after the pinch, and there was no noticeable effect 40 min after the pinch. Similarly, DA uptake inhibition increases, not only striatal extracellular DA levels (16), but also ACh release secondary to the increase in extracellular DA levels. However, further investigations are needed, probably by the use of dopamine receptor antagonists or specfic lesions, to determine if the increased acetylcholine release, due to tail pinch, is an effect mediated solely by dopamine.

Toluene, in contrast to what may be expected from the results mentioned above, decreases striatal ACh release (24). Hence, it has been suggested that toluene exposure decreases striatal ACh release by a DA independent mechanism (24). In the present study, the increase in ACh release induced by tail pinch was not demonstrably affected by a simultaneous exposure to toluene. This result indicates that the dopaminergic influence on striatal ACh release is unaffected by toluene exposure. It, thus, seems that acute toluene exposure affects neither the D_1 nor the D_2 receptor, and supports the interpretation that the toluene induced decrease in basal striatal ACh release (24) is not related to functional changes in DA neurotransmission. The results also suggests that toluene exposure does not affect the striatal acetylcholine response to an acute stressful stimulus.

ACKNOWLEDGEMENTS

The author is grateful to Mikael Darvelid for expert technical assistance. This work was supported by grants number 84-1288, 87-1527 and 89-0096 from the Swedish Work Environment Fund, Stockholm, Sweden.

REFERENCES

1. Ajima, A.; Nakagawa, T.; Kato, T. Simultaneous measurement of acetylcholine and dopamine releases in rat striatum under freely moving conditions with a brain dialysis method. J. Chromatogr. 494:297–302; 1989.
2. Ajima, A.; Yamaguchi, T.; Kato, T. Modulation of acetylcholine release by D_1, D_2 dopamine receptors in rat striatum under freely moving conditions. Brain Res. 518:193–198; 1990.
3. Antelman, S. E.; Szechtman, H.; Chin, P.; Fisher, A. E. Tail pinch-induced eating, gnawing and licking behaviour in rats: Dependence on the nigrostriatal dopamine system. Brain Res. 99: 319–337; 1975.
4. Arlien-Söborg, P. Solvent Neurotoxicity. Boca Raton, FL: CRC Press, Inc.; 1992.
5. Bertorelli, R.; Consolo, S. D_1 and D_2 dopaminergic regulation of acetylcholine release from striata of freely moving rats. J. Neurochem. 54:2145–2148; 1990.
6. Boutelle, M. G.; Zetterström, T.; Pei, Q.; Svennson, L.; Fillenz, M. In vivo neurochemical effects of tail pinch. J. Neurosci. Methods 34:151–157; 1990.
7. Celani, M. F.; Fuxe, K.; Agnati, L. F.; Andersson, K.; Hansson, T.; Gustafsson, J.-A.; Battistini, N.; Eneroth, P. Effects of subacute treatment with toluene on central monoamine receptors in the rat. Reduced affinity in [^{3}H]5-Hydroxytryptamine binding sites and in [^{3}H]-Spiperone binding sites linked to dopamine receptors. Toxicol. Lett. 17:275–281; 1983 .
8. Cenci, M. A.; Kalén, P.; Mandel, R. J.; Björklund, A. Regional differences in the regulation of dopamine and noradrenaline release in medial frontal cortex, nucleus accumbens and caudate-putamen: A microdialysis study in the rat. Brain Res. 581:217–228; 1992.
9. Consolo, S.; Fu Wu, C.; Fiorentini, F.; Ladinsky, H.; Vezzani, A.; Determination of endogenous acetylcholine release in freely moving rats by transtriatal dialysis coupled to a radioenzymatic assay: Effect of drugs. J Neurochem. 48:1459–1465; 1987.
10. Damsma, G.; Westerink, B. H. C.; de Vries, J. B.; Van der Berg, C. J.; Horn, A. S. Measurement of acetylcholine release in freely moving rats by means of automated intracerebral dialysis. J. Neurochem. 48:1523–1528; 1987,.
11. Damsma, G.; de Boer, P.; Westerink, B. H. C.; Fibiger, H. C. Dopaminergic regulation of striatal cholinergic interneurons: An in vivo microdialysis study. Naunyn Schmiederbergs Arch. Pharmacol. 342:523–527; 1990.
12. von Euler, G.; Fuxe, K.; Hansson, T.; Agnati, L. F.; Benfenati, F.; Gustafsson, J.-A. Effects of subacute toluene exposure on protein phosphorylation levels in rat frontoparietal and striatal membranes. Acta Physiol. Scand. 131:113–118; 1987.
13. von Euler, G.; Fuxe, K.; Hansson, T.; Agnati, L. F.; Benfenati, F.; Gustafsson, J.-A. Neurotensin modulates the binding characteristics of dopamine D_2 receptors in rat striatal membranes also following treatment with toluene. Acta Physiol. Scand. 135:443–448; 1989.
14. Finkelstein, Y.; Koffler, B.; Rabey, J. M.; Gilad, G. M. Dynamics of cholinergic synaptic mechanisms in rat hippocampus after stress. Brain Res. 343:314–319; 1985.
15. Gilad, G. M.; Mahon, B. D.; Finkelstein, Y.; Koffler, B.; Gilad, V. H. Stress-induced activation of the hippocampal cholinergic system and the pituitary-adrenocortical axis. Brain Res. 347:404–408; 1985.
16. Hurd, Y. L.; Ungerstedt, U. In vivo neurochemical profile of dopamine uptake inhibitors and releasers in rat caudate-putamen. Eur. J. Pharmacol. 116:251–260; 1989.
17. Imperato, A.; Puglisi-Allegra, S.; Casolina, P.; Zocchi, A.; Angelucci, L. Stress-induced enhancement of dopamine and acetylcholine release in limbic structures: Role of corticosterone. Eur. J. Pharmacol. 165:337–338; 1989.
18. Lehmann, J.; Langer, S. Z. The striatal cholinergic interneuron: Synaptic target of dopaminergic terminals? Neuroscience 10: 1105–1120; 1983.
19. Maysinger, D.; Herrera-Marschitz, M.; Carlsson, A.; Garofalo, L.; Cuello, A. C.; Ungerstedt, U. Striatal and cortical acetylcholine re-

lease in vivo in rats with unilateral decortication: Effects of treatment with monosialoganglioside GM1. Brain Res. 461:355-360; 1988.
20. Nilsson, O. G.; Kalén, K.; Rosengren, E.; Björklund, A. Acetylcholine release in the rat hippocampus as studied by microdialysis is dependent on axonal impulse flow and increases during behavioural activation. Neuroscience 36:325-338; 1990.
21. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1982.
22. Pei, Q.; Zetterström, T.; Fillenz, M. Tail pinched-induced changes in the turnover and release of dopamine and 5-hydroxytryptamine in different brain regions of the rat. Neuroscience 35: 133-138; 1990.
23. Stengård, K.; Höglund, G.; Ungerstedt, U. Extracellular dopamine levels within the striatum increase during inhalation exposure to toluene as recorded by microdialysis in awake, freely moving rats. Toxicol. Lett. 71:245-255; 1994.
24. Stengård, K. Effect of toluene inhalation on extracellular striatal acetylcholine release studied with microdialysis. Pharmacol. Toxicol. 75:115-118; 1994.
25. Stoof, J. C.; Drukarch, B.; de Boer, P., Westerrink, B. H. C.; Groenewegen, H. J. Regulation of the activity of striatal cholinergic neurons by dopamine. Neuroscience 47:755-770; 1992.
26. Ungerstedt, U.; Herrera-Marschitz, M.; Jungelius, U.; Ståhle, L.; Tossman, U.; Zetterström, T. Dopamine synaptic mechanisms reflected in studies combining behavioural recordings and brain release. In: Kotisaka, M.; Shomori, T.; Tsukada, Y.; Woodruff, G. M., eds. Advaces in dopamine research. Oxford: Pergamon Press; 1982:219-231.
27. Westerrink, B. H. C. Sequence and significance of dopamine metabolism in the rat brain. Neurochem. Int. 2:221-227; 1985.
28. Zetterström, T.; Sharp, T.; Ungerstedt. U. Effect of dopamine D_1 and D_2 receptor selective drugs on dopamine release and metabolism in rat striatum in vivo. Naunyn Schmiedebergs Arch. Pharmacol. 334:117-124; 1986.