Acta Neurobiol. Exp. 2002, 62: 93-98

Behavioral sensitization to amphetamine induced by a single i.p. dose of oxotremorine in the rat

Sławomir Gralewicz

Nofer Institute of Occupational Medicine, Department of Toxicology and Carcinogenesis, 8 Teresy St., 90-95 Łódz, Poland, Email: gralslaw@imp.lodz.pl

Abstract. Earlier experiments have revealed that rats treated with a single dose of chlorphenvinphos (CVP), an irreversible acetylcholinesterase inhibitor, are hyposensitive to amphetamine (AMPH) given three weeks after CVP. Exposure to CVP results in an excess of acetylcholine with subsequent overactivation of the nicotinic as well as muscarinic cholinergic receptors. The purpose of the present experiment was to find out whether a selective activation of muscarinic receptors could induce behavioral hyposensitivity to AMPH. To attain this purpose, male rats were pretreated once with 0. 00, 0.135, 0.27 or 0.55 mg/kg of oxotremorine, a muscarinic agonist, and challenged 15 days later with 1.0 mg/kg dose of AMPH. The pre- and postinjection open-field behavior of the rats was tested with the use of a computerized set of activity meters. The testing revealed that in oxotremorine pretreated animals the behavioral response to AMPH, i.e. increase in the ambulatory activity, was not diminished but, to the contrary, it was augmented. This effect was dose-dependent, being most pronounced in rats given the 0.55 mg/kg of oxotremorine. The possible cause of the difference between the effect of CVP and oxotremorine is discussed.

Key words: oxotremorine, behavioral sensitization, amphetamine, open field, rat

The nervous system is the primary target for the action of many xenobiotics. It is known that, in some instances, repeated or even single exposure to neuroactive substances may result in persistent or permanent functional changes within the nervous system (Robinson and Becker 1985, Antelman et al. 1991, 1997). The changes may manifest themselves in the form of altered reactivity (behavioral, vegetative or neuroendocrine) to pharmacological challenges.

In some earlier experiments (Gralewicz et al. 2000) we have found that in the rat, three weeks after a single i.p. dose (1.0 mg/kg (about 1/10 of the LD_{50}) of chlorphenvinphos (CVP), an organophosphorous pesticide, the locomotion stimulating effect of 1.0 mg/kg of d-amphetamine (AMPH) was significantly reduced. CVP is an irreversible acetylcholinesterase (AChE) inhibitor. The observed hyposensitivity to AMPH might thus develop as a result of an excessive activation of cholinergic receptors due to an excess of acetylcholine (ACh). The question is: which of the two main classes of cholinergic receptors, the nicotinic or the muscarinic ones, play the main role in this effect. Nicotinic receptors are unlikely candidates; after exposure to nicotine, rats show an increased rather than decreased behavioral sensitivity to AMPH (Birrell and Balfour 1998). On the other hand, no data are available on the effect of muscarinic agonists on the behavioral responsiveness to AMPH. Oxotremorine (OX) is a selective muscarinic agonist used frequently in behavioral research. The purpose of the present experiment was to detect possible change in the responsiveness of the rat to the locomotor stimulating effect of AMPH after a single exposure to OX.

28 male Wistar rats, outbreeds, from the Institute's breeding colony were used in the experiment. The rats were 4 months old and weighed 280 – 380 g at the experiment onset. For two weeks before the start of the experiment and during the experiment they were housed in single rat cages at 22^{o}C, with a light/dark cycle of 12/12 h (light on at 0600 h). Standard rat food pellets (Murigran) and tap water were accessible *ad libitum*. The rats were divided into four groups: group C, group OX1, group OX2 and group OX3, each consisting of seven animals.

The following chemicals were used for the experiment: oxotremorine ((1-[4-(1-Pyrrolidinyl)-2-butynyl] -2-pyrrolidinone) Sigma), amphetamine (d-amphetamine sulfate, Sigma), and physiological saline (SAL - 0.9% natrium chloratum, Polfa). Before use, OX and AMPH salts were dissolved in bidistilled water to appropriate concentrations. All solutions were administered intraperitoneally at 1ml/kg.

The rat motor activity was assessed with the use of a computerized 4-unit set of activity chambers (PROFEX Ltd, Bialystok, Poland). The set was located in a room, neighboring the animal rooms, and illuminated with white luminescence bulbs located at the ceiling. The ambient temperature and humidity inside the testing room were the same as in the animal rooms. Each activity chamber consisted of clear acrylic open field box (63 x 63 x 40 cm) with 2 tiers of infrared motion sensors spaced 2.5 cm apart. The first and second tier of sensors were 4.0 cm and 15.0 cm from the cage floor. Each cage was equipped with a calculating system, which transformed the beam interruptions into the location of the animal within the cage 5 times per second. Raw data were stored in the cage memory. After the end of a test session the cage memory content was downloaded to a computer memory and subjected to further analysis with the aid of a computer program. A state with no beam interruptions for at least 1 s was classified as "rest". Horizontal shifts of the rat's body equal or longer than 4 cm were regarded as ambulatory movements. Shifts shorter than 4 cm were regarded as nonambulatory (short-distance) movements. Interruption of at least one beam of the upper tier of sensors was counted as a rearing episode.

All parts of the experiment were performed between 0700 and 1500 h. The rats were adapted to the test chambers 1 hour a day for two successive days. Then the effect of OX on the behavioral responsiveness to the selected drug challenges was assessed in three test sessions denoted as session 0, 1 and 2. Each of the test session consisted of two parts, the preinjection part and the postinjection one, each lasting 50 min. After completion of the preinjection part, the rat was transferred to its home cage and the activity chamber was thoroughly cleaned. Then the rat was injected and placed again in the activity chamber for the postinjection testing. The interval between the preinjection and the postinjection testing was 8 - 10 min.

In session 0 and 2 the rats were challenged with 1 ml/kg of SAL. In session 1 they were challenged with 1.0 mg/kg of AMPH. Session 0 was performed 2-3 days before the injection of OX. Sessions 1 and 2 were performed on day 15 and 17, respectively, after the treatment with OX. OX was administered at the following doses: 0.135 mg/kg (group OX1), 0.275 mg/kg (group OX2) or 0.55 mg/kg (group OX3). Rats of the C group

were given SAL. Immediately after the treatment the rat was placed again in its home cage and left undisturbed.

The challenge dose of AMPH was the same as that used in our previous studies (Gralewicz et al. 2000). The doses of OX were established in a pilot experiment. Acute effects of the selected doses of OX on the rat locomotor activity are shown in Fig. 1. The figure shows that OX given intraperitoneally, dose-dependently inhibited the rat locomotor activity. At the dose of 0.55 mg/kg the inhibitory effect was severe and was accompanied by tremor and vegetative symptoms: salivation, micturiction and diarrhea. At 0.135 mg/kg, the effect on locomotion was insignificant and the vegetative symptoms were barely noticeable.

The following indices of the rat behavior in the activity chambers were analyzed: the number of ambulatory (long-distance) movements (AM), the distance covered during ambulation (DIS), the number of nonambulatory, short-distance movements (NAM), and the number of rearings (R). Each of the above indices showed a high individual variability. For example, in the preinjection part of session 0, the AM values ranged from 19 to 112, the DIS values from 15.6 to 97.7 m, the NAM values from 235 to 504, and the R values from 10 to 118. Much lesser variability was noted in individual

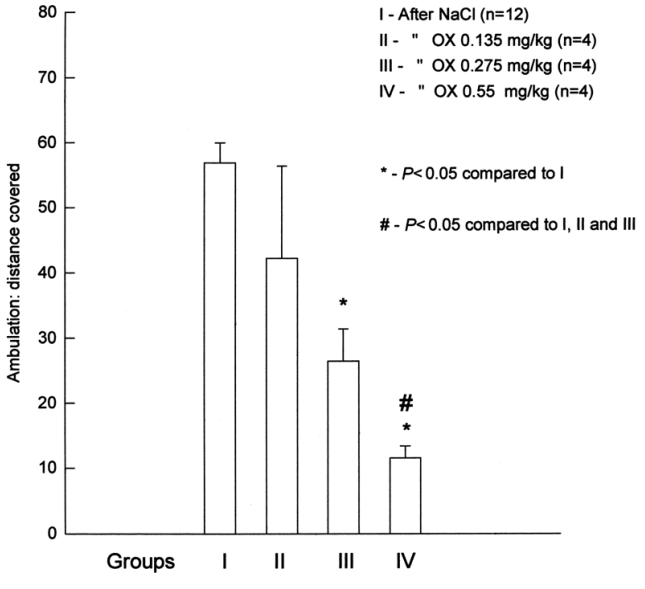

Fig. 1. A diagram illustrating the effect of i.p. injections of oxotremorine at doses used in the experiment on the rat locomotor activity. The bars represent means and SEM of the distance covered by the rat during the 50 min postinjection recording period expressed in percent of the distance covered during the 50 min preinjection recording period.

subject scores obtained in the preinjection parts of successive test sessions; rats scoring high in session 0 did so in the remaining two sessions. Therefore, for statistical comparisons, each direct individual postinjection score was expressed as percent of the respective preinjection one. A two way ANOVA (groups x sessions) was employed. In cases of a significant interaction effect, it was followed by one-way ANOVA and Tukey's test for pairwise comparisons (Winer 1962). Differences were regarded as significant when the probability of the null hypothesis was 5% or less.

In case of each measurement, the effect of the "session" factor and the group x session interaction were significant: (DIS- session effect: $F_{1,23}$=29.94, P<0.0001, group x session interaction: $F_{3,23}$=19.11, P<0.00001. AM – session effect: $F_{1,23}$=21.67, P<0.001, group x session interaction: $F_{3,23}$=15.64, P<0.00001. R – session effect: $F_{1,23}$=14.82, P<0.001, group x session interaction: $F_{3,23}$=6.63, P<0.005. NAM – session effect: $F_{1,23}$=10.62, P<0.005, group x session interaction: $F_{3,23}$=6.91, P< 0.002).

In session 0, the mean DIS, AM and R postinjection values were lower than the preinjection ones in all groups. The NAM value showed no change. In no case was a difference between groups found.

In session 1, in all groups injection of AMPH resulted in a large and, compared to session 0 and 2, statistically significant increase of the DIS and AM values. The AM value, however, was increased to a lesser extent than the DIS value. In other words, after AMPH the distance covered during one ambulation episode was larger than before the injection. Comparisons between groups revealed that the increases in the DIS and AM values were significantly larger in group OX3 than in group C and group OX1 (Fig. 2 A and B). The R values were also increased in the postinjection part of session 1 and this increase was most pronounced in groups pretreated with OX (Fig. 2 C). In the case of this measure, however, the within-group variability was large which made the results of the parametric ANOVA unreliable. Additional comparisons with the use of the Kruskall-Wallis nonparametric one-way ANOVA (Siegel 1956) revealed significant differences (X^2=8.177, df=3, P<0.05); in group OX3 the postinjection increase of the R value was significantly higher than in group C.

In session 1, the postinjection NAM values were decreased in most subjects. This effect was most evident in group OX3; only in this group the differences between sessions were statistically significant ($F_{2,46}$=5.37,

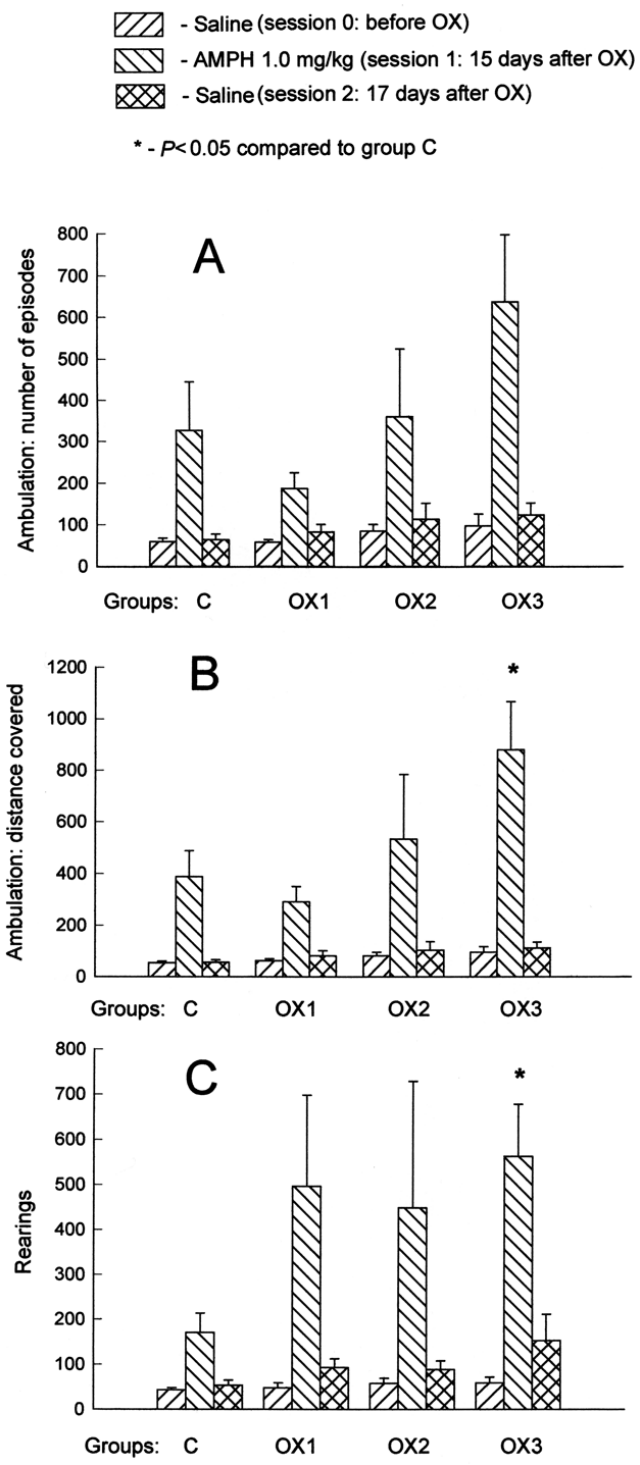

Fig. 2. Diagrams illustrating the effect of 1.0 mg/kg dose of amphetamine on the motor activity of rats pretreated 15 days earlier with oxotremorine. The activity was measured for 50 min before and 50 min after the injection of AMPH. A, number of ambulation episodes; B, total distance covered; C, number of rearings. The bars represent means and SE of the postinjection values expressed in percent of the preinjection ones.

$P<0.01$). Between group comparison revealed no differences.

In session 2, no differences between groups were found.

The results presented above demonstrate that a single administration of OX can sensitize in a dose-related manner rat locomotor behavior to the stimulating effect of AMPH given 15 days after the OX pretreatment. The exploratory behavior is also sensitized by OX; in that case, however, the dose-effect relationship is less evident.

As the literature data show, a long-lasting behavioral sensitization to AMPH may be induced, apart from AMPH itself, by other substances: psychostimulants, opiates, and cannabinoids (Robinson and Becker 1986, Pierce and Kalivas 1997, Vanderschuren et al. 1999). A common feature characterizing many (but not all) of these substances is an ability to activate, directly or indirectly, the dopaminergic system and induce pleasurable emotions. Judging from the effects of systemic administration of muscarinic antagonists, the muscarinic system exerts an inhibitory control over, rather than activating, the dopaminergic system, at least at the level of striatum (Chapman et al. 1997, Tsukada et al. 2000). Moreover, the acute behavioral and vegetative symptoms seen in the rat given muscarinic agonists like OX leave no doubt that the sensations experienced by the animal are aversive rather than rewarding. Yet, as the results of the present experiment show, OX is able to induce behavioral sensitization to AMPH, much like AMPH itself can do (Vandershuren et al. 1999).

It is known that a long-term behavioral sensitization to AMPH can be induced also by physical or psychological stressors. As the existing data suggest, this effect is related somehow with the stressor-induced increase in the level of glucocorticoids (Deroche et al. 1992, 1995, Roberts et al. 1995, Reid et al. 1998). An increase in the level of glucocortiocoids has been also implicated in the induction of behavioral sensitization by psychostimulants, like AMPH (Rivet et al. 1989) and cocaine (Przegalinski et al. 2000). OX stressfulness is clearly manifested in the behavioral symptoms produced by this drug. It is also known that in the rat OX injections result in a dose-dependent increase in the level of plasma corticosterone and that this effect is related with a central action (Steiner and Grahame-Smith 1980). Thus, the augmented behavioral sensitivity to AMPH after the pretreatment with OX in the present experiment may be a consequence of the OX induced stress response, i.e. activation of the hypothalamo-pituitary

-adrenocortical axis. However, exposure to CVP also induces a transient increase in the plasma corticosterone level (Osicka-Koprowska 1984), but three weeks after the exposure the rat appeared hyposensitive to AMPH (Gralewicz et al. 2000). A possible cause of this difference between the effect of CVP and OX may be the difference in the duration of the acute effect. The duration of the OX action may be counted in hours (Hammer et al. 1968). On the other hand, after a single 1.0 mg/kg dose of CVP, the increased ACh level, and hence the overstimulation of the cholinergic receptors, persist for a much longer time, since the restitution of the brain AChE activity requires at least several days (Gralewicz and Soćko 1997). The longer activating pressure on the cholinergic receptors may bring about neuroadaptations qualitatively differing from those induced by a single dose of OX. It cannot be excluded then, that if the time of stimulation by OX was prolonged by applying this drug in multiple, appropriately spaced doses, the change in the sensitivity to AMPH would go in the same direction as after the single dose of CVP. This supposition will be checked in the next experiment.

This work was supported by Grant PB 0726/PO5 /2000/18 from the State Committee for Scientific Research.

Antelman S.M., Caggiula A.R., Kocan D., Knopf S., Meyer D., Edwards D.J., Barry III H. (1991) One experience with "lower" or "higher" intensity stressors, respectively enhances or diminishes responsiveness to haloperidol weeks later: implications for understanding drug variability. Brain Res. 566: 276-283.

Antelman S.M., Soares J.C., Gershon S. (1997) Time-dependent sensitization – possible implications for clinical psychopharmacology. Behav. Pharmacol. 8: 505-514.

Birrell C.E., Balfour D.J. (1998) The influence of nicotine pretreatment on mesoaccumbens dopamine overflow and locomotor responses to D-amphetamine. Psychopharmacology 37: 1503-1513.

Chapman C.A., Yeomans J.S., Blaha C.D., Blackburn J.R. (1997) Increased striatal dopamine efflux follows scopolamine administered systemically or to the tegmental pedunculopontine nucleus. Neuroscience 76: 177-186.

Deroche V., Piazza P.V., Casolini P., Maccari S., Le Moal M., Simon H. (1992) Stress-induced sensitization to amphetamine or morphine psychomotor effects depend on stress-induced corticosterone secretion. Brain Res. 598: 343-348.

Deroche V., Marinelli M., Maccari S., LeMoal M., Simon H., Piazza P.V. (1995) Stress-induced sensitization and glucocorticoids. I. Sensitization of dopamine-dependent locomotor effects of amphetamine and morphine depends on stress-induced corticosterone secretion. J. Neurosci. 15: 7181-7188.

Gralewicz S., Soćko R. (1997) Persisting behavioural and electroencephalographic effects of exposure to chlorphenvinphos, an organophosphorous pesticide, in laboratory animals. Int. J. Occup. Med. Environ. Health 10: 375-394.

Gralewicz S., Lutz P., Szymczak W. (2000) Hyposensitivity to amphetamine following exposure to chlorphenvinphos – protection by amphetamine preexposure. Acta Neurobiol. Exp. 60: 203-207.

Hammer W., Karlen B., Rane A., Sjoquist F. (1968) Rate of metabolism of tremorine and oxotremorine in rats and mice. Life Sci. 7: 197-204.

Osicka-Koprowska A., Lipska M., Wysocka-Paruszewska B. (1984) Effects of chlorphenvinphos on plasma corticosterone and aldosterone levels in rats. Arch. Toxicol. 55: 68-69.

Pierce R.C., Kalivas P.W. (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res. Brain Res. Rev. 25: 192-216.

Przegaliński E., Filip M., Siwanowicz J., Nowak E. (2000) Effect of adrenalectomy and corticosterone on cocaine-induced sensitization in rats. J. Physiol. Pharmacol. 51: 193-204.

Reid M.S., Ho L.B., Tolliver B.K., Wolkowitz O.M., Berger S.P. (1998) Partial reversal of stress-induced behavioral sensitization to amphetamine following metyrapone treatment. Brain Res. 783: 133-142.

Rivet J.M., Stinus L., LeMoal M., Mormede P. (1989) Behavioral sensitization to amphetamine is dependent on corticosteroid receptor activation. Brain Res. 498: 149-153.

Roberts A.J., Lessov C.N., Philips T.J. (1995) Critical role for glucocorticoid receptors in stress- and ethanol-induced locomotor sensitization. J. Pharmacol. Exp. Ther. 275: 790-797.

Robinson T.E., Becker J.B. (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration; a review and evaluation of animal models of amphetamine psychosis. Brain Res. 396: 157-198.

Siegel S. (1956) Nonparametric statistics for the behavioral sciences. McGraw Hill, New York.

Steiner J.A., Grahame-Smith D.G. (1980) Central pharmacological control of corticosterone secretion in the intact rat. Demonstration of cholinergic and serotoninergic facilitatory and alpha-adrenergic inhibitory mechanisms. Psychopharmacology 71: 213-217.

Tsukada H., Harada N., Nishiyama S., Ohba H., Kakiuchi T. (2000) Cholinergic neuronal modulation alters dopamine D2 receptor availability *in vivo* by regulating receptor affinity induced by facilitated synaptic dopamine turnover:

positron emission tomography studies with microdialysis in the conscious monkey brain. J. Neurosci. 20: 7067-7073.

Vanderschuren L.J., Schoffelmeer A.N., Mulder A.H., De Vries T.J. (1999) Dopaminergic mechanisms mediating the long-term expression of locomotor sensitization following preexposure to morphine or amphetamine. Psychopharmacology (Berl) 143: 244-253.

Vanderschuren L.J., Schmidt E.D., DeVries T.J., Van Moorsel C.A., Tilders F.J., Schoffelmeer A.N. (1999) A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. J. Neurosci. 19: 9579-9586.

Winer B.J. (1962) Statistical principles in experimental design. McGraw Hill, New York.

Received 16 November 2001, accepted 27 March 2002