Pharmacology Biochemistry and Behavior, Vol. 62, No. 1, pp. 81–87, 1999

0091-3057/99 $–see front matter

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PII S0091-3057(98)00117-8

Rat Locomotion and Release of Acetylcholinesterase

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Received 17 June 1997; Revised 29 May 1998; Accepted 29 May 1998

HEILAND, B. AND S. A. GREENFIELD. *Rat locomotion and release of acetylcholinesterase.* PHARMACOL BIOCHEM BEHAV **62**(1) 81–87, 1999.—In the substantia nigra acetylcholinesterase is released from the dopamine cells of the pars compacta independent of cholinergic transmission. In this study the effects of local and systemic amphetamine treatment were compared on acetylcholinesterase release in the rat substantia nigra in relation to concomitant behavior. Acetylcholinesterase release, measured "on-line" with a sensitive chemiluminescent system, was enhanced by amphetamine stimulation administered locally and could not be dissociated from simultaneous amphetamine-induced circling behavior. On the other hand, amphetamine administered systemically resulted in a general increase in locomotor behavior followed by a subsequent increase in acetylcholinesterase release. The alternative scenario of an initial rise in acetylcholinesterase release, subsequently followed by enhanced movement, was never seen. Hence, movement can enhance release of acetylcholinesterase from the substantia nigra, whereas "upstream" local nigral events can affect acetylcholinesterase release and movement simultaneously. © 1998 Elsevier Science Inc.

Acetylcholinesterase Amphetamine Substantia nigra Circling behavior Locomotor activity

IT is now well established that in the substantia nigra there are large amounts of acetylcholinesterase (AChE), but very little of its normal substrate acetylcholine (4,5). Moreover, AChE is released in a soluble form from the dendrites of dopaminergic nigrostriatal neurons (7,10,11,27), where it appears to have a subsequent action independent of hydrolysis of acetylcholine (4,5). This phenomenon occurs in the substantia nigra of a wide variety of species, namely of the cat (8), rat (7,27), guinea pig (1,23), and rabbit (11): moreover, this AChE release is calcium dependent (7), and occurs almost exclusively from dopamine-containing cells (7). Several observations have indicated that there may be a close association between AChE and dopamine in the substantia nigra (5). Moreover, there are similarities in the release mechanisms of these two substances in that they are both tetrodotoxin resistant and require calcium entry into the cell [see (6)]. Indeed, application of AChE has been shown to enhance the chronic release of dopamine from the nigrostriatal pathway resulting in a corresponding modification in motor behavior (12–14). It has been postulated that many of the behavioral effects of amphetamine are related to alterations in dopaminergic transmission. Amphetamine acts mainly by blocking the dopamine transporter and, therefore, inhibits the dopamine reuptake and increases the concentration of dopamine at the synapse (21). Behavioral sensitization has been associated with an enhanced dopamine release in the nucleus accumbens and in striatum (22). Striatal efferent neurons are known to be under the influence of dopamine. Current experimental evidence identifies dopamine as the key neurotransmitter in the control of motor activity including circling behavior (20).

In this study, we have attempted to discover whether general increase in movement, as caused by systemic amphetamine, could also cause AChE release and/or whether evoked AChE release, evoked by local perfusion of amphetamine, in turn caused movement. A sensitive chemiluminescent system (24) was used to detect release of AChE while behavior was monitored simultaneously with a special computer system (Antrak-video based animal tracking system).

METHOD

Preparation of Animals

Male Wistar rats, weighing 250–280 g, were anesthetized with Equithesin, 2 ml/kg (12), and placed in a stereotaxic

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frame. A push–pull cannula was implanted unilaterally into the left or right substantia nigra at stereotaxic coordinates [modified from Paxinos and Watson, (19)]: 5.3 mm anterio to bregma; 2.0 mm lateral or dorsal to the midline, and 8.0 mm below the skull. The cannula was secured in place with dental cement to four anchoring screws on the skull. All animals had a light-emitting diode (LED) attached to the implant fixed in place using dental cement. Animals were allowed a minimum of 48 h to recover from surgery, prior to experimentation. All surgically operated animals were housed separately in secure cage-top holding boxes with raised lids to avoid entanglement of the implant. Food and water were available ad lib.

Perfusion via Push–Pull Cannula

The substantia nigra was perfused with artificial cerebrospinal fluid (ACSF) at 37°C, gassed with 95% O_2, 5% CO_2, at a flow rat of 20 μl/ml. The ACSF contained (in mM): NaCl, 127; KCl, 3; $NaHCO_3$, 18.5; KH_2PO_4, 0.6; Na_2HPO_4, 0.5; $CaCl_2$, 2.5; $MgCl_2$, 0.8 and d-glucose, 5. A stock solution of *d*-amphetamine–sulphate (10^{-2} M) was prepared in ACSF and diluted accordingly in ACSF prior to its introduction into the system. *d*-Amphetamine (10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M) was introduced for 5-min periods to the substantia nigra via the cannula and a 15-min recovery period was allowed between consecutive applications.

Assay for Monitoring the Endogenous Release of AChE

AChE activity in the perfusate from the substantia nigra was continuously measured by an "on-line" chemiluminescent assay (24,25) adapted from the original system of Israel and Lesbats (15). Briefly, the assay utilizes the ability of AChE to hydrolise acetylcholine, yielding choline and acetate. Choline is subsequently oxidized to betaine and hydrogen peroxide by choline oxidase. In the presence of hydrogen peroxide peroxidase enzymes, luminol is oxidized and photons are emitted, the amount of light produced being proportional to the amount of AChE present in the perfusate. Prior to the start of each experiment, a calibration curve of known concentrations of exogenous AChE was determined, thus allowing the levels of AChE release in vivo to be calculated. The animals were then connected to the "on-line" system, where the spontaneous release of AChE in the substantia nigra was monitored prior to, during and after stimulation with amphetamine, either locally in the substantia nigra, or systemically by IP injection (*d*-amphetamine dissolved in saline, 1 mg/kg). In addition to animal behavior being recorded on video tape, the AChE-derived light signal was recorded on the audio channel of a second video recorder. Compensation for the delay time had to be performed, because measurement of AChE release occurs ex situ. This delay time was readily calculated by perfusing a bolus of AChE via the implanted push–pull cannula at the end of each experiment. The video and audio signals could then be synchronized so that measurement of AChE release was essentially "on-line" (16,25).

Determination of Animal Movement

Spontaneous or induced animal movements during the perfusion period, were monitored in addition to the video tape using an Antrak-video based animal tracking system (B. Reece Scientific Ltd., Newbury, UK), which was used in conjunction with the "on-line" chemiluminescent technique. This system involves computerised tracking of a LED in and around a preset area within the study arena. The computer then plotted the animals movements within a predetermined time period. The animals movement were also count as a number of 360° turns.

Analysis of Data

Perfusate levels of AChE, both basal and evoked by drug administration, were obtained by measurement of the chemiluminescent signal on an oscilloscope as the area under the curve. These values were subsequently converted into absolute concentrations to quantify release (16,25). Basal levels of AChE were expressed as mU of AChE activity (where 1U of AChE will hydrolyze 1 μmol of acetylcholine per min, at pH 8 and 37°C). Evoked release of AChE was expressed as a percentage of the average value of basal release measured for a 2-min period before and after stimulation. Control values were obtained in a similar manner in the presence of drug vehicle (ACSF) alone. Animal rotation was measured as the number of 360° turns and using the Antrak video-based animal tracking system; motor activity was measured in terms of total distance moved. All results are given as means ± SEM for experiments performed upon *n* animals. Statistical significance of the difference between means was estimated using paired *t*-test. The probability levels interpreted as statistically significant were $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

Histology

At the end of the experiments, to assess cannula placements, animals were deeply anesthetized with halothane (Rhône Mérieux Limited, Harlow Essex, UK) and decapitated. The brains were carefully removed and stored in formaldehyde (4% v/v in phosphate buffered saline, pH 7.4, 4°C) for at least 1 week. Following fixation, brains were placed in cryoprotective solution (30% sucrose in PBS, pH 7.4) until they sink. Sections (42 μm) were cut on a freezing microtome and each section was mounted on a gelatinisated glass slide and stained with cresyl violet.

Materials

The chemicals used in the chemiluminescent assay were all purchased from Sigma Chemical Co., UK: acetylcholinesterase (type VI-S), choline oxidase, acetylcholine chloride, microperoxidase, peroxidase, 5-amino-2,3-dihydro-1,4-phthal-azinedione (luminol).

RESULTS

A total of 30 animals are used in this study, but some animals were not included in the final analysis due to technical problems, for example, blocked push–pull cannula or aberrant cannula placements. Cannula placements were found to be accurately placed in the substantia nigra of rats that circled following local infusion of amphetamine, whereas those with aberrant placements did not circle and were discarded.

Effect of Local Administration of Amphetamine Into the Substantia Nigra

Local administration of amphetamine: behavioral effect. Each concentration of amphetamine was infused over 5 min in ACSF. To compare directly the effects of the different concentrations of amphetamine in the substantia nigra, all concentrations were infused under the same conditions: the circling behavior was measured at the point when amphetamine arrived in the substantia nigra for 15 min to ensure the same condi-

tions for every concentration. Paired *t*-test revealed a significant difference between very modest basal circling, 0.062 ± 0.021 SEM turns/min and 10^{-7} M, 0.22 tuns/min ± 0.05 SEM ($p < 0.05$); 10^{-6} M, 0.28 turns/min ± 0.09 SEM ($p < 0.05$); 10^{-5} M, 0.22 turns/min ± 0.04 SEM ($p < 0.01$); 10^{-4} M, 0.25 turns/min ± 0.05 SEM ($p < 0.01$); 10^{-3} M, 0.23 turns/min ± 0.02 SEM ($p < 0.001$), and 10^{-2} M, 1.11 turns/min ± 0.31 SEM ($p < 0.01$) (see Fig. 1). The highest circling was seen following 10^{-2} M amphetamine with 3.8 turns/min. The turns/min observed with this concentration are significantly different compared with the lower concentrations ranging from 10^{-7} M to 10^{-3} M amphetamine.

Local infusion of one substantia nigra with amphetamine resulted in a characteristic behavior pattern (see Fig. 2); the animals often prefered one region of the box, displaying contraversive circling and exhibiting a very tight rotation around the axis of their body. Amphetamine caused an overall increase in activity of the animals; contraversive behavior circling was seen following all amphetamine concentrations, but with a different pattern at 10^{-2} M amphetamine. By 10^{-2} M amphetamine a high intensity circling was seen, and in some cases animals showed a rapid and persistent shaking of the head: animals showed an extreme twisting of the head, touching the rear flank, and rapid continuous locomotion in one direction. At the end of the amphetamine stimulation, after about 15 min, circling behavior and general activity in movement ceased. This decline in activity coincided with a decrease in the release of AChE.

Local administration of amphetamine: effect on AChE release. Spontaneous hydrolysis of acetylcholinechloride yielded a signal prior to addition of AChE from the rat perfusate. However substraction of this background reading gave the basal AChE perfusate value of 0.25 ± 0.07 mU ($n = 13$). On stimulating the animals with amphetamine (10^{-7} M to 10^{-2} M) administered locally to the substantia nigra, there was an increase in the release of AChE (from 10^{-7} M to 10^{-4} M) with increasing concentrations of amphetamine. By 10^{-5} M to 10^{-4} M however, the increase in AChE release had reached a plateau (see Fig. 3). Amphetamine at 10^{-7} M caused a significant enhancement of 22.87% ($p < 0.01$) in the release of AChE; 10^{-6} M 35.34%, $p < 0.01$; 10^{-5} M 32.44%, $p < 0.05$, and 10^{-4} M 32.91%, $p < 0.01$. The loss of the signal at 10^{-3} M and 10^{-2} M was not attributely to an accumulation of the serial amphetamine doses. These highest concentrations were also tested separately with and without the animal attached to the system, and a loss of the chemiluminescent signal was produced nonetheless. Hence, this reduction in light signal is not due to a physiological inhibition of AChE release, but rather represents a direct chemical "quenching" of the chemiluminescent signal. The same inhibitory effect has been reported with 10^{-6} M 5-HT (5,7-dihydroxytryptamine creatinine sulphate), α-methyl 5-HT (α-methylserotonin maleate), and 2-methyl 5-HT (2-methylserotonin maleate) (2,3).

By local administration of amphetamine we could see a correlation between AChE release in the substantia nigra and behavior measured as turns/min (see Fig. 4). More turns/min

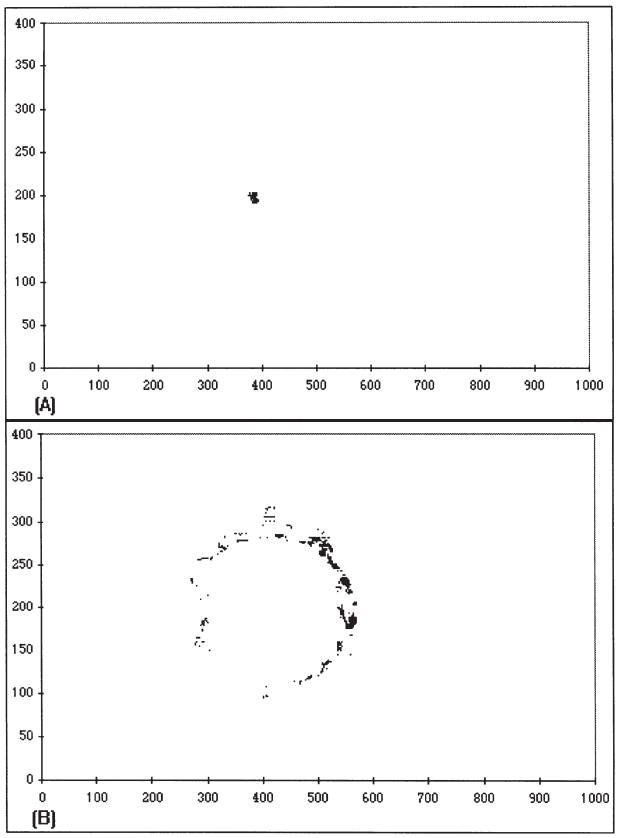

FIG. 2. (A) Animal movement prior to infusion with amphetamine, monitored with an Antrak video-based animal tracking system. Computer plotted picture, total distance moved: 771 mm, time elapsed: 120 s. (B) Animal movement induced by local stimulation (in the substantia nigra via push–pull cannula) with amphetamine, monitored with an Antrak video-based animal tracking system. Computer-plotted picture, total distance moved: 4378 mm, time elapsed: 120 s, amphetaminedose: 10^{-4} M.

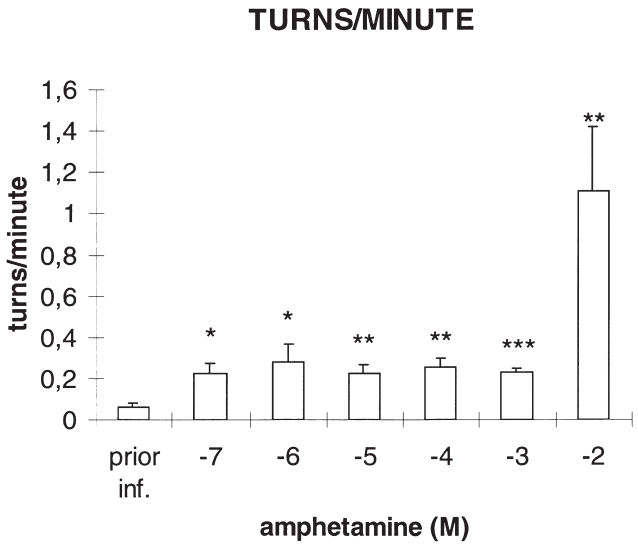

FIG 1. Turns/minute exhibited during 15 min following each of the six consecutive amphetamine concentrations infused into the substantia nigra via push–pull cannula and prior to infusion, with ACSF only. Results are expressed as means ± SEM; asterisks represent a significant difference compared to levels prior to amphetamine; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; paired *t*-test, number of animals used for each concentration are 20.

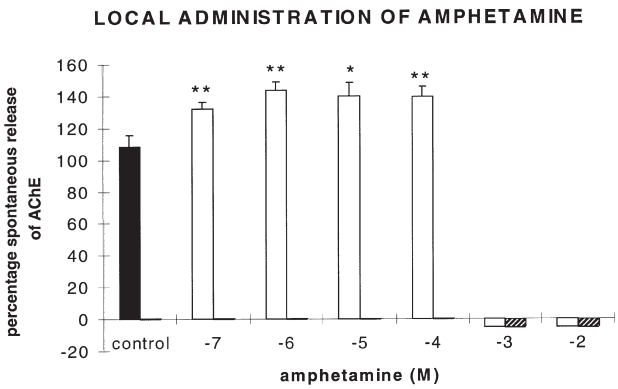

FIG 3. Spontaneous release of AChE in control and amphetamine-treated rats in the substantia nigra expressed as a percentage. Results are shown as means ± SEM; asterisks represent a significant difference from the drug-free control group; $*p < 0.05$, $**p < 0.01$, paired *t*-test, $n = 13$; black column: control group, white column: drug-treated animals, hatched column: with no animal connected to the chemiluminescent system. Note that chemical inhibition of the chemiluminescent signal by 10^{-3} M and 10^{-2} M amphetamine is not due to a physiological inhibition of AChE release, but rather represent a direct chemical "quenching."

correspond to more release of AChE; higher concentration of amphetamine cannot influence turns/min, but has an influence on AChE release in the substantia nigra.

Effect of Systemic Administration of Amphetamine

Systemic administration of amphetamine: behavioral effect. Amphetamine (in saline) was injected IP and the rats tested immediately for enhanced motor activity, using the computer system to monitor total distance moved in millimeters: the same procedure was carried out with control animals, who were injected with saline vehicle only. The computer system detected the movement with help of the LED in the animals headset and showed at which piont (x and y) the animal traveled in terms of distance (mm) and time (in s). The sum of the distance traveled gave the total distance moved in a time period of 120 s. Paired *t*-tests showed a significant difference between control groups and amphetamine stimulation ($p < 0.001$). Mean total distance moved was as follows: treatment with saline only 2787 mm ± 322 SEM, treatment with amphetamine 7493 mm ± 406 SEM, and after stimulation 4617 mm ± 785 SEM (see Fig. 5).

By contrast, systemic stimulation of the animal with 1-mg amphetamine/kg IP produced a different pattern of behavior from that seen with local administration. Typically, approximately 5 min after amphetamine injection the animals become more active, moving around the entire box, lasting for about 1 h in either a contraversive or ipsiversive direction, with a bias varying from animal to animal (see Fig. 6). This increased activity was associated with an increase in release of AChE.

Systemic administration of amphetamine: effects on release of AchE. AChE release was continuously monitored in relation to specific movements evoked by amphetamine stimulation. Spontaneous release of AChE of 0.11 ± 0.03 mU ($n = 9$) was detected in perfusate of the substantia nigra. Application of amphetamine (1 mg/kg) caused a rise in the spontaneous release of AChE of approximately 40% over control conditions ($p < 0.01$, paired *t*-test; see Fig. 7). There was no increase seen with injection of saline only. Increased motor activity was associated with an increase in release of AChE. The raised concentration of AChE release lasted for approximately 1 h at a steady level. The diminution in movements was associated with a decrease in AChE release.

Even by systemic administration of amphetamine we could see a correlation between AChE release in the substantia nigra and behavior measured as total distance moved (see Fig. 8). A rise of released AChE lead to a rise of distance moved and vice versa.

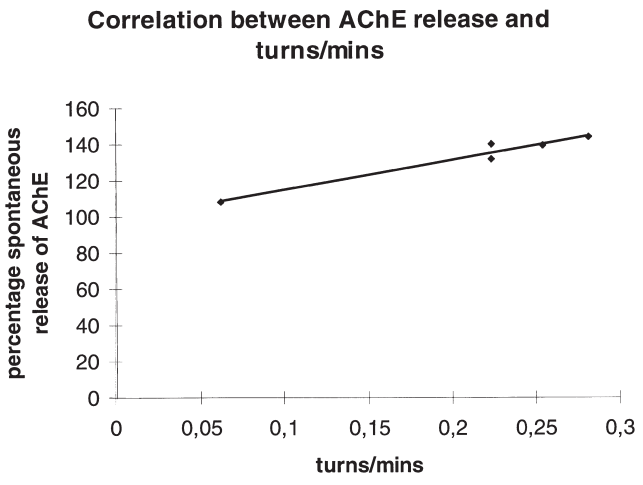

FIG 4. Correlation between AChE release and turns/mins; control: 108% AChE, 0.06 turns/mins; 10^{-7} M amphetamine: 132% AChE, 0.22 turns/min; 10^{-6} M amphetamine: 143% AChE, 0.28 turns/min; 10^{-5} M amphetamine: 139% AChE, 0.22 turns/min; 10^{-4} M amphetamine: 139% AChE, 0.25 turns/min.

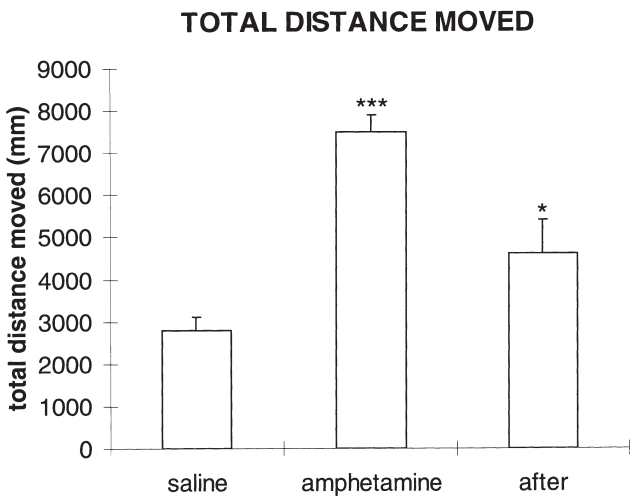

FIG. 5. Total distance moved (mm) during systemic stimulation with amphetamine (1 mg/kg) compared to control groups injected with saline only, and approximately 1 h later after amphetamine stimulation. Results are expressed as means ± SEM; asterisks represent a significant difference from drug-free control group, $*p < 0.05$, $***p < 0.001$, paired *t*-test, $n = 10$.

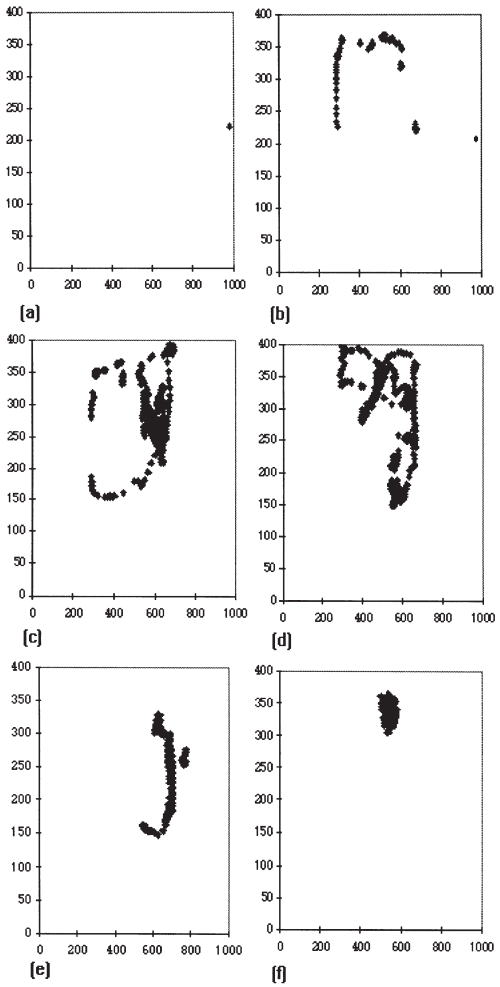

FIG. 6. Cumulative data showing motor activity after IP treatment with saline (a); with amphetamine (b–e), and approximately 1 h after amphetamine treatment (f). The behavior of the first 480 s evoked by amphetamine stimulation is shown and approximately 1 h later. The animal movement induced by systemic stimulation with amphetamine is monitored with an Antrak video-based animal tracking system. Different computer plotted pictures are shown in a time of 120 s each. Total distance moved (in mm): (a) 788, (b) 3572, (c) 5268, (d) 6484, (e) 3421, (f) 2129.

DISCUSSION

In recent years the "on-line" chemiluminescence technique has been used for the determination of AChE activity in the guinea pig substantia nigra (1,23). The assay has been shown to be sensitive and reliable for detection of the release of AChE in vivo (24). AChE release from the substantia nigra and striatum has been also demonstraded in several studies in the rat (7,26,27). Moreover, Jones and Greenfield (17) showed that AChE release increased when the animal was moving. Indeed, both in electrophysiological experiments in vivo (18) and in behavioral studies (9,12,13,14,26), exogenous AChE has a facilitating action on the net activity of the nigrostriatal pathway that is not seen following administration of butyrylcholinesterase. Secretion of AChE could be viewed, therefore, as an important component in the cellular basis of motor control in the nigrostriatal system.

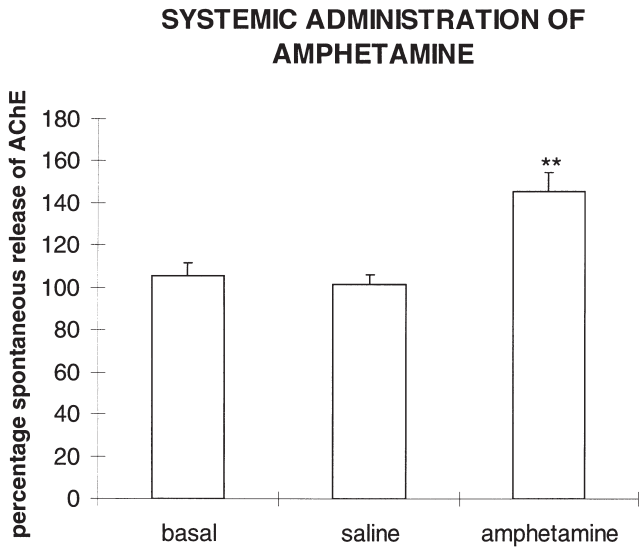

FIG. 7. Spontaneous release of AChE from substantia nigra shown as basal, and in control and in animals treated with systemic amphetamine. Results are expressed as means ± SEM; asterisks represent significant difference from the drug-free control group, $^{**}p < 0.01$, paired t-test, $n = 9$.

AChE release evoked in the substantia nigra by local amphetamine stimulation was enhanced with increasing concentration of amphetamine infused in the substantia nigra 22–35% over control, reaching a plateau by 10^{-5} and 10^{-4} M. An even greater enhancement of the concentration of AChE following amphetamine in the substantia nigra was not possible. In any event, the enhanced AChE release was correlated with enhanced circling behavior, which, nontheless, remained at a minimal difference of intensity. A different type of circling

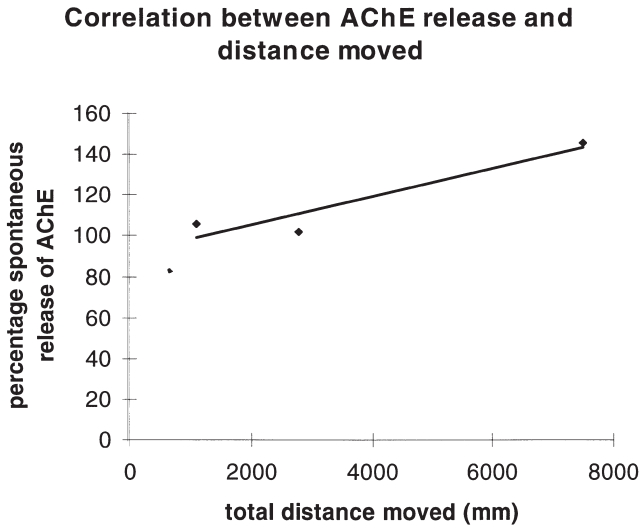

FIG. 8. Correlation between AChE release and total distance moved (mm); basal: 105% AChE, 1085 mm distance moved; control: 101% AChE, 2787 mm distance moved; application of amphetamine IP: 145% AChE, 7492 mm distance moved; $r^2 = 0.878$.

was seen by 10^{-2} M, compared to the weaker concentrations of amphetamine. This tighter type of rotation could be the result of stimulation of adjacent brain regions, for example, VTA (ventral tegmental area), to where the stronger concentration of amphetamine could gain access by passive diffusion.

In the present experiment circling behavior was never seen without enhanced AChE release and vice versa. Therefore, we conclude that AChE release in the substantia nigra result from the action of amphetamine, but circling behavior results from the action of a higher concentration of AChE in the substantia nigra.

Systemically administered amphetamine treatment immediately enhanced the AChE release 40% over control conditions in the substantia nigra and at the same time enhanced the motor activity with a bias relative to control animals. Pycock (20) has pointed out that rats and other rodents show a natural preference to one particular side, and that this preference asymmetry can be revealed as turning behavior following systemic administration of dopamine agonists such as amphetamine. However, amphetamine stimulation systemically as given here, will stimulate not only the nigral pathway but other regions as well, such as the mesolimbic pathway, and indeed other cerebral systems indirectly stimulated by dopamine.

The increase in AChE release following systemic amphetamine administration was always subsequent to the onset of locomotor behavior. Amphetamine inhibits the reuptake of dopamine and, therefore, the increased concentration of dopamine caused locomotor behavior and an increase in AChE release.

In both cases, as for local as well as systemic stimulation with amphetamine, there is a correlation between AChE released and behavior, the higher the motor activity of the animals the higher the release of AChE. We cannot say enhanced mobility triggers AChE or enhanced concentration of AChE in the substantia nigra triggers mobility, because both phenomenons could observe at the same time.

In conclusion, these results show that AChE release in substantia nigra is closely associated with dopamine modulating behavior. This neurochemical phenomenon appears to be mainly the result of movement rather than a cause: however, once released, it can further enhance movement in a feedforward mechanism. Because the dopamine cells of the substantia nigra play an important role in the pathophysiology of Parkinson's disease, these findings may be of value in furtherance our understanding of the neurochemical mechanisms involved in the operatives of this key neuronal population.

ACKNOWLEDGEMENTS

BH was funded by Human Capital and Mobility. We would like to thank Ms. L. Guiver for processing the histology, Mrs. L. Annetts for photography, and Dr. C. Hawkins for her statistical advice.

REFERENCES

1. Dally, J. J.; Greenfield, S. A.: The release of acetylcholinesterase in vivo is regulated by dopaminergic systems in the guinea-pig substantia nigra. Neurochem. Int. 25:339–344; 1994.
2. Dickie, B. G. M.; Greenfield, S. A.: Raphé nucleus-evoked release of acetylcholinesterase from guinea-pig substantia nigra. Neurochem. Neuroreport 5:769–772; 1994.
3. Dickie, B. G. M.; Greenfield, S. A.: Release of acetylcholinesterase from guinea-pig substantia nigra: Effects of tryptaminergic drugs and dorsal raphé nucleus stimulation. Neuropharmacology 34:1191–1200; 1995.
4. Greenfield, S. A.: A noncholinergic action of acetylcholinesterase (AChE) in the brain: From neuronal secretion to the generation of movement. Cell. Mol. Neurobiol. 11:55–77; 1991.
5. Greenfield, S. A.: Novel neuronal mechanisms in the normal and Parkinsonian substantia nigra. In: Adaption and aging. New York: Academic Press Ltd.; 1995:203–214.
6. Greenfield, S. A.: The significance of dendritic release of transmitter and protein in the substantia nigra. Neurochem. Int. 7:887–901; 1985.
7. Greenfield, S. A.; Chéramy, A.; Glowinski, J.: Evoked release of proteins from central neurons in vivo. J. Neurochem. 40:1048–1056; 1983.
8. Greenfield, S. A.; Cheramy, A.; Glowinski, J.: In vivo release of acetylcholinesterase in cat substantia nigra and caudate nuclei. Nature 284:355–357; 1980.
9. Greenfield, S. A.; Chubb, I. W.; Grünewald, R. A.; Henderson, Z.; May, J.; Portnoy, S.; Weston, J.; Wright, M. C.: A non-cholinergic function for acetylcholinesterase in the substantia nigra: Behavioural evidence. Exp. Brain Res. 54:513–520; 1984.
10. Greenfield, S. A.; Shaw, S. G.: Release of acetylcholinesterase and aminopeptidase in vivo following infusion of *d*-amphetamine into the substantia nigra. Neuroscience 7:2883–2893; 1982.
11. Greenfield, S. A.; Smith, A. D.: The influence of electrical stimulation of certain brain regions on the concentration of acetylcholinesterase in rabbit cerebrospinal fluid. Brain Res. 177:445–459; 1979.
12. Hawkins, C. A.; Greenfield, S. A.: Non-cholinergic action of exogenous acetylcholinesterase in the rat substantia nigra. I. Differential effects on motor behaviour. Behav. Brain Res. 48:153–157; 1992.
13. Hawkins, C. A.; Greenfield, S. A.: Non-cholinergic action of exogenous acetylcholinesterase in the rat substantia nigra. II. Long-term interactions with dopamine metabolism. Behav. Brain Res. 48:159–163; 1992.
14. Hawkins, C. A.; Greenfield, S. A.: Recombinant acetylcholinesterase has behavioural effects in the rat substantia nigra not attributable to its enzymatic activity. Neurosci. Lett. 197:203–206; 1995.
15. Israel, M.; Lesbats, B.: Chemiluminescent determination of acetylcholin and continuous detection of its release from Torpedo electric organ synapses and synaptosomes. Neurochem. Int. 3:81–90; 1981.
16. Jones, S. A.; Annetts, C.; Preston, M.; Greenfield, S. A.: An improved method for correlating behaviour with acetylcholinesterase release from the brain of the awake guinea-pig "on-line." J. Physiol. 430:62; 1990.
17. Jones, S. A.; Greenfieldm S. A.: Behavioural correlates of the release and subsequent action of acetylcholinesterase secreted in the substantia nigra. Eur. J. Neurosci. 3:292–295; 1990.
18. Last, A. T. J.; Greenfield, S. A.: Acetylcholinesterase has a noncholinergic neuromodulatory action in the guinea-pig substantia nigra. Exp. Brain Res. 67:445–448; 1987.
19. Paxinos, G.; Watson, C.: The rat brain in stereotaxis coordinates. New York: Academic Press; 1982.
20. Pycock, C. J.: Turning behaviour in animals. Neuroscience 5:461–514;1980.
21. Robinson, T. E.; Becker, J. B.: Enduring changes in brain and behaviour produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev. 11:157–198; 1986.
22. Robinson, T. E.; Jursonm P. A.; Bennett, J. A.; Bengten, K. M.: Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+) amphetamine: A microdialysis study in freely moving rats. Brain Res. 462:211–222; 1988.
23. Taylor, S. J.; Bartlett, M. J.; Greenfield, S. A.: Release of acetyl-

cholinesterase within the guinea pig substantia nigra: Effects of 5-hydroxytryptamine and amphetamine. Neuropharmacology 27: 507–514; 1988.
24. Taylor, S. J.; Haggblad, J.; Greenfield, S. A.: Measurement of cholinesterase activity released from the brain "on-line" and in vivo. Neurochem. Int. 15:199–205; 1989.
25. Taylor, S. J.; Jones, S. A.; Haggblad, J.; Greenfield, S. A.: "On-line" measurement of acetylcholinesterase release from the substantia nigra of the freely-moving guinea-pig. Neuroscience 37:71–76; 1990.
26. Weston, J.; Greenfield, S. A.: Application of acetylcholinesterase to the substantia nigra induces stereotypy in rats. Behav. Brain Res. 18:71–74; 1985.
27. Weston, J.; Greenfield, S. A." Release of acetylcholinesterase in the rat nigrostriatal pathway: Relation to receptor activation and firing rate. Neuroscience 17:1079–1088; 1986.