PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 442–443 (1976)

The Effect of Dopamine on the Electrically Induced Release of [^{3}H]Choline from Rat Striatal Slices[1] (39230)

NANCY PERKINS AND THOMAS C. WESTFALL

Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22903

It is well established that the mammalian caudate nucleus has both dopaminergic and cholinergic innervation (1, 2). The cell bodies of the dopaminergic neurons are found mainly in the pars compacta of the substantia nigra and appear to project mainly to the caudate nucleus and putamen (3). Unlike the dopaminergic neurons, some cholinergic fibers appear to exist as interneurons with both cell bodies and terminals present within this structure (4, 5). Recently it has been suggested that dopaminergic fibers make synapses with cell bodies of the cholinergic interneurons in the caudate nucleus (4, 6, 7). These studies have also suggested that dopamine exerts an inhibitory effect on cholinergic interneurons. For instance, blockade of dopaminergic transmission with neuroleptic drugs increases the turnover of acetylcholine in striatal neurons (6) and decreases striatal acetylcholine levels. This neuroleptic-induced increase of acetylcholine turnover can be prevented or reversed by dopamine receptor stimulating agents such as apomorphine or L-DOPA (6, 7) and these same agonists produce an increase striatal levels of acetylcholine (8).

The present studies were carried out to investigate whether or not exogenously administered dopamine could alter the release of acetylcholine induced by electrical stimulation of superfused slices of rat striatum. It was thought that this would provide information concerning the concept of inhibitory dopaminergic regulation of cholinergic neurotransmission in this structure.

Methods and materials. Rats were killed by decapitation and brains quickly removed and placed in the cold room at 4° where they were sliced with a MacIlwain tissue chopper at a thickness of 1 mm. Individual striatal slices of this thickness and approximately 3 cm in diameter were then separated from other brain regions. They were placed in 1.9 ml of Krebs–Ringer buffer at 37° for 10 min. The buffer had the following composition in mM; NaCl, 126.5; $NaHCO_3$, 27.5; KCl, 2.4; KH_2PO_4, 0.5; $CaCl_2$, 1.1; $MgCl_2$, 0.83; Na_2SO_4, 0.5; glucose, 5.9.

The slices were incubated with 10 μCi of [^{3}H]choline (sp act 17 Ci/mmole) for 40 min in an atmosphere of 95% O_2–5% CO_2. Following the incubation, individual slices were placed in specially designed glass chambers and held in place between two platinum mesh grids which also served as electrodes (10, 11). The slices were then superfused with physiological buffer at a rate of 1.0 ml/min with Buchner peristaltic pumps. The total volume of the chambers was 2.0 ml and the entire unit was jacketed so that water could be circulated at 37°. An initial 40-min superfusion with normal buffer was followed by an additional 40-min superfusion with buffer containing $10^{-4}M$ hemicholinium (Aldrich). Various concentrations of dopamine (10^{-7}–10^{-5} M) were added to the superfusion medium 10 min prior to electrical stimulation of the slices. Ascorbic acid was present in the buffer to protect the catecholamine from oxidation. The superfusate effluent was continuously collected at various intervals during the entire experiment.

After 80 min (40 min with normal buffer and 40 min with a buffer containing hemicholinium) the slices were stimulated for 2 min with biphase square wave pulses (100 pps; 1.1 msec duration; 5 V) by means of two Grass stimulators. Each slice was stimulated only once. The wave form, intensity, and duration of the electrical puses was continuously monitored by a Ev-70A Heath dual trace oscilloscope. [^{3}H]choline in the perfusate effluent was determined by liquid-scintillation spectrometry in a manner described by Szerb and Somogyi (11).

[1] Supported in part by U.S. Public Health Service Grant No. 10260, The Council for Tobacco Research, USA, and GM 02139 Training Grant.

Copyright © 1976 by the Society for Experimental Biology and Medicine
All rights reserved.

A statistical comparison between the release of [^{3}H]choline from control slices with the release from slices treated with dopamine was made utilizing Student's *t* test.

Results. The effect of various concentrations of dopamine added to the superfusion medium on the electrically induced release of labeled choline is shown by Fig. 1. It was observed that a concentration of 10^{-5} *M* dopamine did not have a statistically significant effect on the release of [^{3}H]choline. At concentrations of 10^{-6} and 10^{-7} *M*, however, dopamine produced a significant suppression of the electrically induced release of [^{3}H]choline ($P < 0.05$).

Discussion. Szerb and Somogyi (11) have utilized this method of introducing labeled choline in isolated tissues. They have presented evidence showing that labeled choline is incorporated into newly synthesized acetycholine and that this newly synthesized ^{3}H-mediator is affected by the same conditions known to effect acetylcholine. The increased efflux of [^{3}H]choline was blocked by tetradotoxin and abolished by omission of extracellular calcium from the medium. From this and other data, the authors conclude that [^{3}H]choline originates from ^{3}H-stores that do not exchange with choline in other compartments because hemicholinium, which is present during superfusion, blocks such an exchange. This appears, therefore, as a valid method of accessing the release of acetylcholine and is devoid of the problem of utilizing an anticholinesterase, which results in the production of high levels of endogenous acetylcholine. This could influence the release of acetylcholine by an action on presynaptic cholinergic receptors.

In the present study, electrical stimulation produced a measurable increase in [^{3}H]choline, which was also found to be dependent upon extracellular calcium and was reduced by tetrodotoxin. The inhibition of the electrically induced release of [^{3}H]choline by 10^{-6} and 10^{-7} dopamine is consistent with the idea that dopaminergic neurons exert an inhibory influence on the release of acetylcholine from striatal slices. It is of interest that the lower, perhaps more physiological concentrations of dopamine was the most effective in inducing this effect. The present results provide direct evidence for the dopamine inhibitory hypothesis that has been generated with the use of dopamine receptor agonists and antagonists *in vivo* (4, 6–8).

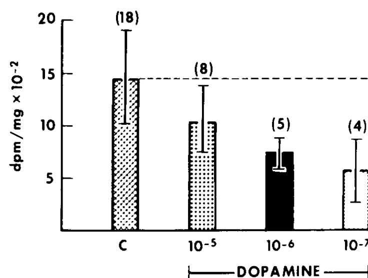

FIG. 1. The effect of electrical stimulatin on the release of [^{3}H]choline from normal slices (c, ■) or from slices in which various concentrations of dopamine had been added to the superfusion medium. Data is plotted as increase in [^{3}H]choline over prestimulation level as dpm/mg tissue ± SEM. Number above each column refers to the number of slices.

1. Vogt, M., Brit. Med. Bull. **29,** 168 (1973).
2. Hornykiewicz, O., Brit. Med. Bull. **29,** 172 (1973).
3. Fuxe, K., Hofkelt, T., and Ungerstedt, V., Int. Rev. Neurobiol. **13,** 93 (1970).
4. Stadler, H., Lloyd, K. G., and Bartholini, G., Naunynschmiedeberg's Arch. Pharmacol. **283,** 129 (1974).
5. Guyenet, P., Agid, Y., Javoy, F., Beaujoan, J. C., and Glowinski, J., C. R. Acad. Sci. (Paris) **278,** 26 (1974).
6. Stadler, H., Lloyd, K. E., Cadea-Ciria, M., and Bartholini, G., Brain Res. **55,** 476 (1973).
7. Bartholini, G., Stadler, H., and Lloyd, K. C., "Frontiers in Catecholamine Research" (S. Snyder and E. Usdin, eds.), pp. 741-745. Pergamon Press, Oxford (1974).
8. Sethy, V. H., and Van Woert, M. H., Nature (London) **251,** 52P (1974).
9. Agid, Y., Guyenet, P., Javoy, F., Beaujoan, J. C., and Glowinski, J., J. de Pharmacologie, **5** Suppl. 1, 59 (1974).
10. Westfall, T. C., Life Sci. **14,** 1641 (1974a).
11. Westfall, T. C., Neuropharmacol. **13,** 693 (1974b).
12. Szerb, J. C., and Somogyi, G. T., Nature New Biol. **241,** 121 (1973).

Received September 18, 1975. P.S.E.B.M. 1976, Vol. 151.