There is, of course, also the possibility that the dopamine receptors in the limbic system are blocked to a greater extent than those in the corpus striatum by clozapine but not by haloperidol. Finally, the results indicate that determinations of HVA in the corpus striatum and in the limbic system of rabbits may be of value in predicting the ability of neuroleptic drugs to induce in man extrapyramidal and antipsychotic actions, respectively.

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Department of Pharmacology,
University of Göteborg, Fack
S-400 33 Göteborg 33, Sweden.

NILS-ERIK ANDÉN
GÜNTER STOCK

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The effect of trivastal, haloperidol and dibutyryl cyclic AMP on [^{14}C]dopamine synthesis in rat striatum

We have previously reported that apomorphine inhibits the biosynthesis of [^{14}C]-dopamine from [^{14}C] tyrosine in striatal slices more effectively than it inhibits tyrosine hydroxylase activity *in vitro* (Goldstein, Freedman & Backstrom, 1970). The effective inhibition of [^{14}C] dopamine biosynthesis in striatal slices by apomorphine could either be due to accumulation of the drug in the dopamine-containing neurons and its subsequent inhibition of tyrosine hydroxylase or to the drug's stimulation of the dopamine receptor resulting in a feed-back control of dopamine biosynthesis. We have now further investigated the effects of activation and blockade of dopamine receptors on [^{14}C] dopamine biosynthesis in striatal slices. For the activation of the dopamine receptors animals were treated with trivastal (1, 2″-pyrimidyl)-4-piperonyl-piperazine: (T495) a recently described dopamine receptor stimulating agent (Corrodi, Fuxe & Ungerstedt, 1971) and for blockade haloperidol was used. Since adenylate cyclase may be the receptor for dopamine in the striatum (Kebabian, Petzold & Greengard, 1972) we have also investigated the effects of dibutyryl cyclic AMP (dB-cAMP) on [^{14}C] dopamine biosynthesis in striatal slices.

Male Sprague-Dawley rats, 250–300 g, were decapitated and the striata immediately dissected, sliced and incubated at 37° in Krebs Henseleit medium. The incubation

Table 1. *The effect of trivastal and/or of haloperidol administration on [^{14}C]dopamine biosynthesis in striatal slices.* The results are the means ± standard errors from four experiments and are expressed as pCi per incubation. Each incubation contained approximately 80 mg of slices. The changes from controls in the experiments below were significant ($P < 0.01$). The net uptake of [^{14}C]tyrosine into the striatal slices was not significantly different in the treated animals as compared with the untreated animals.

Treatment*	[^{14}C]Dopamine in striatal slices	% Change from controls
None	12·5 ± 1·4	
Haloperidol	23·2 ± 1·9	+ 91
Trivastal	7·9 ± 0·9	— 34
Haloperidol * Trivastal **	21·4 ± 1·8	+ 75

* Trivastal (15 mg kg^{-1}, i.p.) or haloperidol (10 mg kg^{-1}, i.p.) were given 1 h before the animals were killed.

** Haloperidol (10 mg kg^{-1}, i.p.) was given 1 h before trivastal (20 mg kg^{-1}, i.p.). The animals were killed 1 h after the administration of trivastal.

procedure and the determination of [^{14}C] catecholamines was as previously described (Goldstein, Ohi & Backstrom, 1970). The substrate [^{14}C] tyrosine (2 μCi; 1 μg) was added to each sample following a pre-incubation period of 10 min. The medium contained the monoamine oxidase inhibitor pargyline, 5×10^{-4}M and the incubation was for 20 min. The test compounds were added to the preincubation medium as indicated in the Tables. Tyrosine hydroxylase activity was measured according to Nagatsu, Levitt & Udenfriend (1964).

Trivastal, like apomorphine, inhibits effectively the biosynthesis of [^{14}C]dopamine from [^{14}C]tyrosine in striatal slices even at 10^{-6}M. The effects of trivastal and/or haloperidol administration on [^{14}C]dopamine synthesis in striatal slices are presented

Table 2. *The effects of dB-cAMP on [^{14}C]dopamine biosynthesis in striatal slices.* The results are the means ± standard errors from at least four experiments and are expressed as pmol per incubation. Each incubation contained approximately 80 mg of slices. The changes from the corresponding controls were significant ($P < 0.01$). The net uptake of [^{14}C]tyrosine into the striatal slices was increased by approximately 15–20% in slices incubated in a medium containing 5×10^{-4}M dB-cAMP.

Treatment*	dB-cAMP**	[^{14}C]Dopamine in striatal slices
None	0·0	14·5 ± 1·1
None	10^{-6}	18·3 ± 1·4
None	5×10^{-4}	29·6 ± 2·7
None	5×10^{-4}***	22·4 ± 1·9
Haloperidol	0·0	24·5 ± 2·3
Haloperidol	5×10^{-4}	31·2 ± 2·8
Trivastal	0·0	8·6 ± 1·0
Trivastal	5×10^{-4}	28·4 ± 2·5

* The animals were treated with haloperidol or with trivastal as indicated in Table 1.

** Theophylline 5×10^{-4}M was added to the incubation medium 5 min before the addition of dB-cAMP.

*** Theophylline omitted.

in Table 1. The blockade of dopamine receptors with haloperidol results in an enhancement while the activation of dopamine receptors by treatment with trivastal results in an inhibition of [^{14}C]dopamine biosynthesis in striatal slices. Treatment of rats with haloperidol antagonizes the trivastal-induced inhibition of [^{14}C]dopamine synthesis.

The effects of dB-cAMP on [^{14}C]dopamine synthesis from [^{14}C]tyrosine in striatal slices are presented in Table 2. Synthesis was stimulated at 10^{-6}M dB-cAMP with maximal stimulation at 5×10^{-4}M. When theophylline was omitted from the incubation mixture, the synthesis was slightly but significantly reduced suggesting that this stimulation is due at least in part, to the cyclic nucleotide component of the molecule. However, it was not prevented by blocking the dopamine receptor with haloperidol or by stimulating it with trivastal.

Unlike apomorphine, trivastal does not contain a catechol group and does not inhibit tyrosine hydroxylase *in vitro*. It is possible that *in vivo* the methylene group of trivastal is cleaved and a metabolite containing a catechol group is formed. However the finding that trivastal inhibits effectively the [^{14}C]dopamine synthesis in striatal slices *in vitro* suggests that the parent compound and not its metabolite exerts the inhibitory activity. Thus, the inhibition of [^{14}C]dopamine biosynthesis by trivastal in striatal slices is probably related to the dopamine receptor stimulating properties of the drug. The finding that the blockade of dopamine receptors by haloperidol antagonizes the trivastal-induced inhibition of [^{14}C]dopamine synthesis further supports the idea that the inhibitory activity of trivastal is related to its receptor stimulating properties.

The formation of [^{14}C]dopamine from [^{14}C]tyrosine in the striatum is accelerated by treatment with neuroleptic drugs (Anden, Corrodi & others, 1967; Nyback & Sedvall, 1971). It was assumed that dopamine synthesis induced by neuroleptics is mediated via an increased flow of nerve impulses. However, as well as earlier findings (Cheramy, Javoy & others, 1972; Kehr, Carlsson & others, 1972) the present observations indicate that the neuroleptic-induced acceleration of dopamine synthesis might not entirely be mediated via an increased flow of nerve impulses. Thus, our studies support the recent report that a receptor mediated feedback regulates the biosynthesis of dopamine in the striatum (Kehr & others, 1972).

That the stimulation of dopamine receptors elevates the activity of adenyl cyclase (Kebabian & others, 1972) and, as we have found, also inhibits the synthesis of dopamine, prompted us to investigate whether dB-cAMP inhibits the synthesis of dopamine in the striatum. The unexpected finding that it elevates rather than inhibits the synthesis of dopamine in the striatum suggests that the effects of the nucleotide are not associated with the changes in the activity of the dopamine receptor. This assumption is also supported by the findings that dB-cAMP elevates the synthesis of dopamine in striatal slices of animals treated with haloperidol or with trivastal. The mechanism by which dB-cAMP elevates the synthesis of dopamine is still obscure. The increase in the net uptake of [^{14}C]tyrosine induced by dB-cAMP could only be partially responsible for the elevation of dopamine synthesis. It should be pointed out that the accumulation of [^{14}C]tyrosine into the brain slices seems not to be the rate-limiting step in catecholamine synthesis (Goldstein & others, 1970). Since depolarizing agents like K^+ ions (Harris & Roth, 1971) or ouabain (Goldstein & others, 1970) elevate the catecholamine synthesis in brain slices and stimulate the formation of cyclic AMP it is conceivable that these two effects are linked. Furthermore, our results indicate that cyclic AMP may be involved in the regulation of catecholamine synthesis.

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New York University Medical Center,
Department of Psychiatry,
Neurochemistry Laboratories,
New York, N.Y. 10016, U.S.A.

M. GOLDSTEIN
B. ANAGNOSTE
C. SHIRRON

January 2, 1973

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Pharmacological studies on a possible role of central noradrenaline neurons in respiratory control

In studies on central noradrenaline vasodepressor mechanisms (Bolme & Fuxe, 1971; Bolme, Corrodi & Fuxe, 1972, 1973) it was discovered that drugs increasing noradrenaline receptor activity in the central nervous system can decrease the respiratory frequency of anaesthetized rats. A short report of the pharmacological evidence suggesting the existence of a noradrenaline mechanism in the neural control of respiration is now given.

Male Sprague-Dawley rats (200-250 g) in groups of 5–6 animals were anaesthetized with 1–1·25% fluothane in oxygen. The body temperature was regularly controlled and if necessary adjusted with a heating lamp. The drug treatments used were: (1) The central noradrenaline receptor stimulating agent, clonidine, (Andén, Corrodi & others, 1970; Bolme & Fuxe, 1971; Schmitt, Schmitt & Fenard, 1971) was given in a dose of 10 μg kg^{-1} (i.v.); (2). Another drug 2,6-dichlorobenzylidene aminoguanidine acetate (DCBAG, Wy 8678, Wyeth Laboratories) also recently shown to be a central noradrenaline receptor stimulating agent (Bolme, Corrodi & Fuxe, 1972, 1973), was given in a dose of 50 μg kg^{-1} (i.v.); (3) L-dopa was given in a dose of 150 mg kg^{-1} (i.p.) 60 min after treatment with a peripheral dopadecarboxylase inhibitor, 1-α-(3,4-dihydroxybenzyl) α-hydrazinopropionic acid (MK 486; 100 mg kg^{-1}, i.p.; Porter, Watson & others, 1962; Bartholini & Pletscher, 1969). This treatment results in increased central dopamine and noradrenaline activity (see Henning & Rubenson, 1970; Rubenson, 1971).

The results are summarized in Table 1. Clonidine caused a 15–20% decrease of respiration rate 5–10 min after the injection. The rate was restored after 30–60 min. DCBAG caused a 30% decrease of the rate, which lasted for 30 min. After 60 min the rate was almost restored to its preinjection value. The combined MK 486-dopa treatment also resulted in a decrease of respiration rate, which was about 20% and started after 15 min. After 60 min the rate was almost back to normal.

The three drug treatments have one thing in common—that they cause increases in central noradrenaline receptor activity. It is therefore possible that a central noradrenaline receptor stimulation is responsible for the decrease of respiration frequency observed. In view of this, a central noradrenergic mechanism may also exist in the neural control of respiration of unanaesthetized rats. This possibility becomes even