both TR length and bristle number were significantly smaller ($p < 0.001$) than in the bilateral animals, while inter-bristle distance was not. A striking observation made in *vestigial* animals was that in males, 14 out of 74 cases produced no TR structures in either wing (18.9%), while in females 26 of 52 failed to do so (50.0%).

Discussion. These data indicate clearly that, while much improvement toward the wild-type phenotype in wing structure is achieved in both sexes in *vestigial* animals raised at 31 °C, wing development is never complete, though it is consistently better in males than in females. This represents a reversal of the condition seen in wild-type flies at any temperature: TR length is routinely 10–15% greater in females than in males, possibly reflecting the generally larger size of female flies. The relationship between bristle number and inter-bristle distance reveals another interesting aspect of wing border development: although bristle number is greatly reduced from the wild-type standard in 31 °C *vestigial* animals, inter-bristle distance does not increase significantly in females, and does so only slightly (6.8 μm) in males ($p < 0.02$). It thus appears that the capacity to construct the TR wing border is expressed in a nearly normal manner by *vestigial* flies if it is expressed at all; a small number of TR bristles will not be spread over a long span of wing border. This observation is certainly in keeping with the expression of other mutations which affect the border of the wing[5,6]. It may also help to explain the apparent lack of regenerative capacity in the *vestigial* wing disc[2].

1 Stanley, W.F., Phys. Zool. *4* (1931) 402.
2 Bownes, M., and Roberts, S., Differentiation *18* (1981) 89.
3 Li, J.C., and Tsui, Y., Genetics *21* (1936) 248.
4 James, A.A., and Bryant, P.J., Devl Biol. *85* (1981) 39.
5 McCrady, E., unpublished data.
6 Fristrom, D., Molec. gen. Genet. *103* (1969) 363.
7 Simpson, P., Lawrence, P.A., and Maschatt, F., Devl Biol. *84* (1981) 206.

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Evidence for an activating effect of tabernanthine on rat brain catecholamine synthesis and elimination[1]

M. Prioux-Guyonneau, E. Mocaër-Cretet, Y. Cohen and C. Jacquot

Laboratoire de Pharmacologie, ERA-CNRS 627, Faculté de Pharmacie, F-92290 Chatenay-Malabry (France), 11 January 1982

Summary. Tabernanthine increased the synthesis and elimination of catecholamines (CA) in the striatum and the rest of the brain, but not in the hypothalamus. These data provide evidence that tabernanthine may activate CA turnover of some brain structures by acting at 2 steps of the metabolic pathway. The results are discussed in relation to a central stimulating action and a hypoxia antagonistic effect of this drug.

Key words. Rat brain; striatum; tabernanthine; catecholamine turnover.

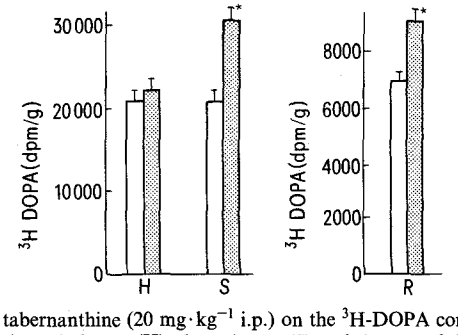
Catecholamine (CA) turnover rate is often calculated by measuring CA efflux after tyrosine hydroxylase (TH) blockade[2]. However, this method only provides information on the effect occurring at the release/degradation step and does not allow us to evaluate exactly the effect of a drug on the CA synthesis. Indeed a drug may act either on both synthesis and degradation or on only one of these steps.

The central stimulating agent, tabernanthine, has temorigenic and weak cataleptic actions[3-6] which were supposed to be related to variations in brain neurotransmitter metabolism. We have previously shown, using α-methyl paratyrosine (α-MT) to inhibit CA synthesis, that tabernanthine slightly decreases the brain turnover time of CA[7]. This effect was assumed to be due mainly to an increase in the monoamine catabolism and/or release. However, an effect on the synthesis could not be ruled out.

The present work was undertaken to study, in parallel, in several brain fractions, the influence of tabernanthine on both the synthesis and elimination processes of norepinephrine (NE) and dopamine (DA). Tyrosine hydroxylase activity was estimated by ^{3}H-DOPA accumulation after ^{3}H-tyrosine (^{3}H-TYR) and DOPA-decarboxylase inhibitor administration. The elimination process was investigated by determining the decrease in NE and DA levels after blockade of their synthesis.

Materials and methods. Experiments were performed on male Sprague-Dawley rats (Ch. River) weighing 200–240 g. The following drugs were used: Tabernanthine tartrate (CNRS, Gif-sur-Yvette, France), hydroxy benzyl hydrazine hydrochloride (NSD 1015) and α-methyl paratyrosine methyl ester (α-MT (SIGMA), L-[3,5-^{3}H] tyrosine (46 Ci·mmol^{-1}) and [3-^{14}C] DOPA (50 mCi·mmol^{-1}) (CEA, France).

^{3}H-DOPA concentration was mesured 65 min after DOPA decarboxylase inhibition by NSD (250 mg·kg^{-1} i.p.) followed 10 min later by ^{3}H-TYR injection (500 μCi·kg^{-1} i.v.). TYR chemical purity was previously verified to check the absence of DOPA. Treated rats received, 10 min before NSD, an i.p. injection of tabernanthine tartrate (20 mg·kg^{-1} as base) and were compared to a control group receiving only ^{3}H-TYR and NSD. The rats were killed by decapitation and brains dissected on a chilled plate into hypothalamus, striatum and rest of the brain. Tissues were weighed and homogenized in ice-cold 0.5 M $HClO_4$. After centrifugation, ^{3}H-DOPA from the supernatant was adsorbed on alumina at pH 8.6, as described by ANTON and SAYRE for catechol compounds[8]. Then ^{3}H-TYR was washed out by 3 additions of distilled water and ^{3}H-DOPA was eluted by twice 5 ml of 1 N HCl. Radioactivity of aliquots of each fraction was estimated by liquid scintillation counting. In order to verify TYR-elimination and to estimate DOPA recovery in the HCl eluates, ^{3}H-TYR and ^{14}C-



Effects of tabernanthine (20 mg·kg^{-1} i.p.) on the ^{3}H-DOPA concentration in the hypothalamus (H), the striatum (S) and the rest of the brain (R) after ^{3}H-tyrosine (500 μCi·kg^{-1} i.v.) and NSD (200 mg·kg^{-1} i.p.) administration. Results are means of 6–8 determinations expressed as dpm·g^{-1} ± SEM of fresh tissue. □, Controls; ▨, treated; *$p < 0.05$ between control and treated.

Effect of tabernanthine (20 mg·kg^{-1} i.p.) on the rate constant of loss and turnover rate of norepinephrine (NE) and dopamine (DA) in various brain areas

		NE		DA	
		Hypothalamus	Rest of the brain	Striatum	Rest of the brain
Rate constant of loss K^{h-1} ± SD	Control	0.153 ± 0.029	0.280 ± 0.027	0.250 ± 0.023	0.215 ± 0.027
	Treated	0.146 ± 0.032	0.473 ± 0.048**	0.357 ± 0.028**	0.333 ± 0.031**
Turnover rate ng·g^{h-1} ± SD	Control	260 ± 58	53.8 ± 7.2	1352 ± 171	31.8 ± 5.6
	Treated	229 ± 57	84.6 ± 12.8*	2109 ± 279*	52.9 ± 7.01*

The rate constant of loss is the slope ± SD of the amine level decrease induced by α-MT (250 mg·kg^{-1} i.p.) during 3 h. The turnover rate is the product ± SD of the initial level by the rate constant of amine decline.
*$p < 0.05$; **$p < 0.01$; significations between control and treated.

DOPA were added in vitro to the brain homogenates in the ratio ^{3}H-TYR/^{3}H-DOPA found in vivo after ^{3}H-TYR injection. These samples and the experimental ones were treated in parallel as described above. The percentage of TYR contamination in the eluate was 0.8 ± 0.2% and DOPA recovery was 80 ± 2%. Results were expressed as dpm·g^{-1} (means ± SEM) of fresh tissues.

The NE and DA levels and the level decreases after inhibition of the synthesis by α-MT were determined in 4 groups of rats receiving: 1) saline; 2) α-MT (250 mg·kg^{-1} i.p.); 3) tabernanthine tartrate (20 mg·kg^{-1} i.p. as base); 4) tabernanthine and 10 min later α-MT. The animals were killed by decapitation 1, 2 or 3 h after each of these treatments. The above described procedures were followed for dissection, homogenization and adsorption on alumina of both catecholamines[8]. Then CA were eluted by 0.05 N $HClO_4$ and measured by the fluorimetric method[9,10]. The slope of the level decrease was calculated by the method of least squares (log of levels versus time) using 4 values for each group: control, 1, 2 and 3 h levels after α-MT. The rate constant of loss (K^{h-1}) was calculated according to Brodie et al.[2] and the SD of the slope according to Schwartz[11]. The turnover rate ng·g^{h-1} is the product of the NE or DA initial level and K ± SD[2].

Results. Effect of tabernanthine on ^{3}H-DOPA accumulation. The amount of DOPA newly synthesized from labeled TYR was significantly increased by tabernanthine in the striatum and the rest of the brain ($p < 0.05$). The radioactivity of these 2 brain fractions rose from 21,500 ± 2900 to 33,700 ± 4400 dpm/g and from 7000 ± 820 to 9200 ± 470 dpm/g, respectively. On the other hand, the hypothalamus concentration of DOPA was not modified by the treatment (21,250 ± 3300 dpm/g in control and 23,900 ± 2500 dpm/g in treated group) (fig.).

Effect of tabernanthine on the rate constant of loss and turnover rate of NE and DA. The decline of NE in the rest of the brain after α-MT(k^{h-1}) was significantly higher in treated rats than in controls (+41%, $p < 0.01$). The NE turnover rate (ng·g^{h-1}) was also increased (+36%, $p < 0.05$). By contrast these 2 parameters were not modified in the hypothalamus (table).

The DA loss was significantly increased by the treatment in the striatum (+32%, $p < 0.01$) and in the rest of the brain (35%, $p < 0.01$). In these 2 structures turnover rate was increased by 36 and 40% (table).

Discussion. It is well known that a drug that does not modify the steady state of the CA level may nevertheless change the turnover of these neurotransmitters by modification of the synthesis and/or elimination[2]. The degree of accumulation of DOPA, after DOPA-decarboxylase blockade, is considered to be a synthesis index while the rate of decrease in NE and DA level after tyrosine-hydroxylase inhibition is an elimination index[2,12].

The present study showed that tabernanthine increased both synthesis and elimination of CA in the striatum and the rest of the brain. Indeed an enhancement of DOPA accumulation was found in these 2 structures together with an increased DA elimination in the striatum and increased DA/NE elimination in the rest of the brain. These observations allow us to rule out an action occurring only at one step of the metabolic pathway and stimulating CA turnover by a feedback mechanism as suggested previously[7]. By contrast, neither DOPA accumulation nor a change in NE release was seen in the hypothalamus. A difference in the distribution of the drug in different brain areas may be suggested to explain this phenomenon. Further pharmacokinetic studies would be necessary to confirm this hypothesis.

The tabernanthine effect in the striatum and the rest of the brain can be related to the antagonistic action of this drug, previously observed in the decrease of CA turnover induced by hypobaric hypoxia, especially in these brain areas[7]: this effect would be consistent with a better O_2 utilization by the oxygen-dependent enzymes involved in CA metabolism. Our results provide, moreover, biochemical support for the pharmacological observation of Zetler[5] who assumed that the mechanism of central stimulating action of tabernanthine is different from that of amphetamine-like compounds. Indeed these latter compounds increase both NE and DA turnover[13], with an important effect on the hypothalamus, while tabernanthine is deprived of activity on this structure. Furthermore, it must be noticed that the tabernanthine effects are not limited to the CA system, since an accelerated turnover of rat brain serotonin in hypoxia[14] and a decrease of GABA level in the mouse brain[15] have been reported elsewere, suggesting that tabernanthine may act on CA metabolism through other neurotransmitter systems.

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2 Brodie, B.B., Costa, E., Dlabac, A., Neff, N.N., and Smookler, H.H., J. Pharmac. exp.Thér. *154* (1966) 493.
3 Raymond-Hamet, M., and Vincent, D., C.r. Soc. Biol. *154* (1960) 2223.
4 Zetler, G., and Lessau, W., Pharmacology *8* (1972) 235.
5 Zetler, G., Arzneimittel-Forsch. *11* (1964) 1277.
6 Prioux-Guyonneau, M., Rapin, J.R., and Wepierre, J., J. Pharmac., Paris *8* (1977) 383.
7 Cretet, E., Prioux-Guyonneau, M., Jacquot, C., Sentenac, H., and Wepierre, J., Naunyn-Schmiedebergs Arch. Pharmak. *313* (1980) 119.
8 Anton, A.H., and Sayre, D.F., J. Pharmac. exp. Thér. *138* (1962) 360.
9 Euler von, U.S., and Lishajko, F., Acta physiol. scand. *51* (1961) 348.
10 Anton, A.H., and Sayre, D.F., J. Pharmac. exp. Thér. *165* (1964) 326.
11 Schwartz, D. Méthodes statistiques à l'usage des médecins et des biologistes, Flammarion, Paris (1963).
12 Carlsson, A., Davis, J.N., Kehr, W., Lindqvist, M., and Atack, C.V., Naunyn-Schmiedebergs Arch. Pharmak. *275* (1972) 153.
13 Estler, C.J., Adv. Pharmac. Chemother. *13* (1975) 305.
14 Prioux-Guyonneau, M., Mocaër-Cretet, E., Redjimi-Hafsi, F., and Jacquot, C., Gen. Pharmac. *13* (1982) 251.
15 Mocaër-Cretet, E., Hajo, N., Dupont, Ch., Jacquot, C., and Wepierre, J., J. Pharmac., Paris *11* (1980) 330.

0014-4754/84/121388-02$1.50 + 0.20/0