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Biochemical Pharmacology, Vol. 31, No. 14, pp. 2430–2432, 1982.
Printed in Great Britain.

0006-2952/82/142430-03 $03.00/0

Effects of the thyroliberin analogue CG 3703 on noradrenergic and serotoninergic transmission in rodents

(*Received* 27 *November* 1981; *accepted* 10 *February* 1982)

The complex behavioural response to thyroliberin (TRH) injections suggests an increased activity of different monoaminergic systems of the central nervous system (for review see [1]). Whereas an enhanced dopamine (DA) release by TRH is well documented [1], some doubts about a relevant involvement of the norepinephrine (NE) system appeared justified, since brain NE levels after TRH application remain unaltered and only slight changes in [^{3}H] NE turnover could be established [2]. Some symptoms in TRH-treated animals, particularly the "wet dog shaking" in rats, indicated an increased serotoninergic transmission [3]. However, any alteration of the 5-hydroxytryptamine (5-HT) system could so far not be verified by biochemical methods. In order to clarify the possible contribution of NE and 5-HT to the TRH syndrome, we reinvestigated the problem by means of the analogue CG 3703 (6-methyl-5-oxo-thiomorpholinyl-3-carbonyl-histidyl-prolinamide). This compound which is resistent against the TRH degrading pyroglutamate aminopeptidases from serum and tissue [4] induces a typical, but prolonged TRH syndrome at minute dosages [3, 5] and thus substantially facilitates the analysis of the biochemical basis of TRH-induced behaviour.

Materials and methods

NE was extracted from brain tissue according to Haubrich and Denzer [6] and determined by the fluorometric method of Weil-Malherbe and Bigelow [7]. NE utilisation was estimated as NE decline in α-methyl-*p*-tyrosine (α-MPT)-treated male NMRI mice of 18–22 g body wt. as described by Brodie *et al.* [8].

5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were determined fluorometrically according to Curzon and Green [9]. 5-HT release was estimated by determination of 5-HIAA accumulation in probenecid-pretreated (200 mg/kg i.p.) male Wistar rats of 150–200 g body wt. 5-HT biosynthesis was evaluated by measuring 5-HT accumulation in animals pretreated with tranylcypromine (10 mg/kg i.p.). Test compound and probenecid or tranylcypromine, respectively, were administered simultaneously and the animals were sacrificed 1 hr later.

Drug effects were analysed for statistical significance by means of the two-tailed Student's *t*-test.

TRH was obtained from Serva GmbH (Heidelberg, F.R.G.). CG 3703 was synthesised [10] by Dr. E. Schwertner, Grünenthal GmbH (F.R.G.).

Results and discussion

CG 3703 did not affect the NE level of the brain at dosages up to 1 mg/kg (Table 1), whereas the ED_{50} values for this compound in various behavioural models were considerably lower [3, 5]. At 10 mg/kg, however, CG 3703 tended to reduce the NE levels in rat brain and significantly decreased the NE content of mouse brain. Mice were

Table 1. Influence of CG 3703 on the NE level in the whole brain of mice and rats

Drugs (mg/kg i.p.)	Species	nmole NE/g	±S.E.	*n*	P
Control (0)	Mouse	1.88	0.05	10	—
CG 3703 (0.5)	Mouse	1.80	0.08	5	n.s.*
CG 3703 (1.0)	Mouse	1.84	0.16	5	n.s.
CG 3703 (10)	Mouse	1.44	0.04	5	<0.001
Control (0)	Rat	2.63	0.17	10	—
CG 3703 (10)	Rat	2.24	0.13	10	<0.1

NE was measured 2 hr after drug administration.
* n.s. = no significant difference vs control.

therefore selected to investigate further the influence on NE turnover by CG 3703. In whole brain of α-MPT-treated mice CG 3703 increased the NE decline in a clearly dose-dependent manner. Starting at a dosage of 0.5 mg/kg CG 3703 i.p., the NE levels differed significantly from those of α-MPT-treated control animals (Fig. 1). It can thus be concluded that CG 3703 effectively enhanced the NE turnover. The high rate of NE utilisation probably reflects an increased rate of NE release from synaptical storage sites. The range of dosages at which CG 3703 thereby enhanced noradrenergic transmission corresponds well with those triggering a variety of pharmacological effects such as stimulation of group motility, enhancement of amphetamine toxicity, antagonism of barbiturate-induced anaesthesia and reserpine hypothermia [5].

Surprisingly, TRH up to 40 mg/kg apparently did not induce any significant change in the NE turnover rate if the NE determination was performed 2 hr after treatment (Fig. 1A). Based on behavioural studies, TRH is only approx. 80 times less active than CG 3703 and therefore some effect on the NE system had to be anticipated. Besides, this finding does not agree with several reports on enhanced NE turnover at 10–40 mg/kg TRH [1, 11–16].

Taking into account the short biological half-life of TRH, we repeated the experiment with a minor modification. The animals were treated with α-MPT, and then with CG 3703 or TRH 1 hr later. Residual NE levels were determined 1 hr after test drug applications (Fig. 1B). The results obtained with CG 3703 were not substantially altered by this experimental modification. However, now the NE levels after 20 and 40 mg TRH also ranged significantly below those of the corresponding α-MPT controls. We conclude from these results that TRH also induces a consistent but very transient increase in NE turnover, which is easily overlooked by the methodology employed, if the block of NE synthesis is still incomplete when TRH reaches the nervous tissue. The TRH effect may also be obscured, if

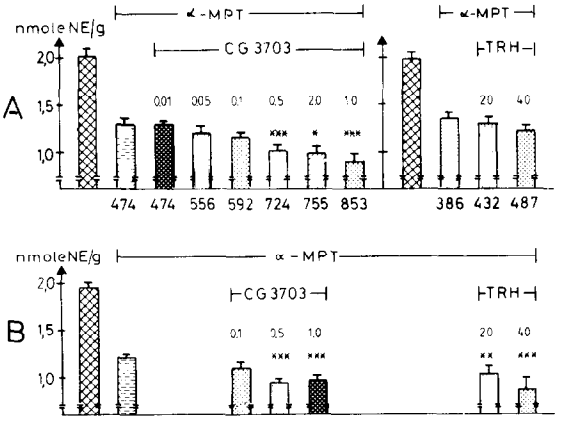

Fig. 1. Brain NE levels of mice before and after inhibition of NE biosynthesis by α-MPT (200 mg/kg i.p.). Influence on NE turnover of CG 3703 and TRH at dosages indicated is deducible from differences in NE levels of animals treated with α-MPT alone and α-MPT plus test compound. Data for Fig. 1A were obtained 2 hr after simultaneous treatment with α-MPT and test compounds. Figure 1B shows corresponding results after consecutive treatment with α-MPT 2 hr before, and test compound 1 hr before, sacrificing the animals. Please note that the NE decline in Figs. 1A and B is not strictly comparable due to different time of exposure to test compounds. * = $P < 0.02$; ** = $P < 0.005$; *** = $P < 0.001$ vs α-MPT control group. Numbers above columns: i.p. dosage of test compounds in mg/kg. Numbers below columns (Fig. 1A): rates of NE turnover in pmole/g/hr. Bars indicate standard error (S.E.).

Table 2. Influence of CG 3703 on levels, biosynthesis and utilization of 5-HT in rat brain

Drugs (mg/kg i.p.)	5-HT ± S.E. (*n*) nmole/g	Rate of 5-HT formation nmole/g/hr	5-HIAA ± S.E. (*n*) nmole/g	Rate of 5-HIAA accumulation nmole/g/hr
Control I (0)	3.92 ± 0.15 (16)	—	—	—
CG 3703 (10)	3.81 ± 0.11 (17) (n.s.)*	—	—	—
Control II (0)	—	—	2.73 ± 0.10 (8)	—
Probenecid (200)	—	—	4.53 ± 0.07 (8)	1.80
Probenecid (200) + CG 3703 (10)	—	—	4.67 ± 0.11 (8)	1.94 (n.s.)
Control III (0)	3.86 ± 0.18 (8)	—	—	—
Tranylcypromine (10)	6.10 ± 0.19 (8)	2.24	—	—
Tranylcypromine (10) + CG 3703 (10)	5.81 ± 0.15 (11)	1.95 (n.s.)	—	—

Determination of 5-HT and 5-HIAA was performed 1 hr after drug administration.
* n.s. = no significant difference *vs* control.

the time chosen for the determination of NE decline is long as compared with the short duration of action of TRH.

In the absence of α-MPT, the increased NE utilisation upon TRH application is obviously fully compensated for by increased biosynthesis, since a fall in NE levels could never be demonstrated [1]. At 10 mg/kg CG 3703, however, the NE utilisation is obviously too much increased to be balanced by resynthesis.

"Wet dog shaking" behaviour is frequently discussed to indicate increased serotoninergic transmission. This behavioural syndrome is consistently observed in rats treated with TRH or its analogues [1, 3, 5]. We therefore investigated whether in this species at least any disturbance of the brain 5-HT turnover could be biochemically substantiated by means of the potent TRH analogue CG 3703.

5-HT levels of rat brain, found unchanged after acute TRH treatment in previous reports [1, 11, 13], were also not affected by the excessive dosage of 10 mg/kg CG 3703 (Table 2). In order to exclude an enhanced, but compensated 5-HT utilisation, *in vivo* biosynthesis and turnover of 5-HT upon CG 3703 exposure were measured independently (Table 2). The rate of 5-HT biosynthesis in the brain was estimated by determining the increase of 5-HT in rats treated with the monoamine oxidase inhibitor tranylcypromine for 1 hr. As shown in Table 2, the 5-HT biosynthesis is not at all affected by CG 3703. Utilised 5-HT was estimated as the acid metabolite 5-HIAA accumulating in the brain of probenecid-treated rats. One hr after probenecid treatment (Table 2) the calculated 5-HT turnover rates for probenecid controls and the CG 3703 group did not differ significantly. We thus were unable to provide any biochemical evidence that the 5-HT system is affected by acute application of 3703. In this context it should be mentioned that the "wet dog shaking" behaviour in TRH-treated rats [1, 3] does not necessarily indicate 5-HT activity. Recently, it has been reported that the TRH-induced "wet dog shaking" behaviour is only partially antagonised by 5-HT antagonists but effectively by several dopamine receptor blockers (haloperidol, chlorpromazine, and pimozide) and propranolol [17].

In conclusion, the degradation-stabilised TRH analogue CG 3703 induces an increased NE turnover at pharmacologically active dosages. Only at excessive dosages do the NE levels decrease, whereas at lower dosages the NE utilisation appears to be balanced by increased NE biosynthesis. The 5-HT system is not affected at any dosage of CG 3703. We therefore have to consider noradrenergic and dopaminergic, but not serotoninergic contributions to the behavioural syndrome triggered by TRH or its analogues.

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