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TRH-induced Behavioral Arousal in Developing Rats Pretreated with 6-Hydroxydopa

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NOMURA, Y. AND K. OKI. *TRH-induced behavioral arousal in developing rats pretreated with 6-hydroxydopa.* PHARMAC. BIOCHEM. BEHAV. 12(6) 925–930, 1980.—The behavioral effect of thyrotropin releasing hormone (TRH) was investigated in the developing rat pretreated with 6-OHDOPA at birth. An IP injection of TRH (20 mg/kg) increased walking with sniffing, rearing, body shaking, grooming, chewing and licking in the 7-, 14-, 20- and 30-day-old as well as in the adult rat. TRH-induced locomotor stimulation began a few minutes after the injection and lasted for approximately 60 min. But on Day 7, TRH produced locomotor stimulation between 1.5 hr and 3.5 hr after the injection. Neonatal treatment with 6-OHDOPA markedly potentiated TRH-induced locomotor stimulation and behavioral arousal in the 7-day-old rat but not in the 14-day-old and adult rat. The marked potentiation of TRH-induced locomotor stimulation by 6-OHDOPA in the 7-day-old rat was reduced by α-flupenthixol (pA_2=5.9) and phenoxybenzamine (pA_2=4.4). These results suggest that central dopamine neurons are involved in TRH-induced behavioral arousal in the infant rat.

TRH 6-OHDOPA Behavioral arousal Developing rat Central catecholamine neurons

THYROTROPIN releasing hormone (TRH), a hypothalamic peptide, potentiates the actions of L-dihydroxyphenylalanine (L-DOPA) in pargyline-treated rats and mice [28,29]. TRH is distributed in brain regions outside of the hypothalamic area [7,34], suggesting a neuromodulating action of TRH in the central nervous system (CNS). The rise in spontaneous locomotor activity following TRH administration in normal rats is related to altered catecholamine and possibly 5-hydroxydopamine (5-HT) metabolism brought about by this hormone [1]. A bilateral microinjection of TRH into the nucleus accumbens of the rat induced locomotor hyperactivity which was blocked by pretreatment with haloperidol and 6-hydroxydopamine (6-OHDA) [6,14]. These results suggest that TRH produces locomotor hyperactivity by acting on the dopamine (DA) system in the nucleus accumbens. However, Kulig [11] reported that TRH does not cause locomotor stimulation in the rat. Furthermore, a recent paper does not support a locomotor stimulant action of TRH but other behavioral actions such as body shaking and limb tremor [4]. Another recent paper reports that catecholaminergic neural systems in the brain are not required for the locomotor stimulant action of TRH [33]. In addition to this discrepancy, there are few studies on the behavioral effects of TRH in the developing rat.

It has been suggested that both DA and noradrenaline (NA) system could become functional at least in certain areas at birth in the rat CNS, since an IP injection of methamphetamine and clonidine results in locomotor hyperactivity in the 1-day-old rat [21], Nomura *et al.*, unpublished observation). Behavioral response to dopaminergic and noradrenergic agents dramatically differs at various developmental stages [3, 9, 12, 13, 18, 31]. This seems to be closely related with the developmental change of the interaction between different transmitter systems and with the regional development of function of the transmitter system. We found that behavioral response to apomorphine and atropine were modified in the developing rat neonatally treated with 6-OHDOPA [18,19]. It is of interest to examine the influence of 6-OHDOPA on TRH-induced behavioral arousal in the rat during postnatal life.

In the present paper, we investigated the behavioral effect of TRH in the developing rat pretreated with 6-OHDOPA at birth, to gain insight into the mechanism of TRH-induced locomotor hyperactivity and the functional development of the central catecholaminergic system.

METHOD

Animals and the Treatment with 6-OHDOPA

Wistar rats of both sexes were used. After birth all litters were culled to 10–12 pups/mother. Either 6-OHDOPA (75 mg/kg), dissolved in saline containing 0.001 N HCl, or the vehicle was first injected subcutaneously (SC) into rats within several hours after birth, followed by SC injections at 2 and 4 days. Treated pups were kept with a mother under daylight conditions at 23°C and were weaned on Day 22.

TABLE 1
TRH-INDUCED BEHAVIORAL AROUSAL IN DEVELOPING RATS

Age (days)	Treatment (mg/kg)	Behavior observed in controls and TRH-treated rats: Walking	Rearing	Head, body & limb shaking	Grooming (scratching)	Chewing	Sniffing	Licking (nibbling)	Straub Tail
7	Control	1–2	0	0	1–2	1	0	0	–
	TRH 20	3 (Crawling)	0	3	3	3	3	5	+
14	Control	2	0	0	1	0	0	0	–
	TRH 20	5	0	5	3	3	4	5	+
20	Control	3–4	3	0	3	0	2	3	–
	TRH 20	5	4	4	4	3	3	4	+
30	Control	3–4	3	0	3	0	2	3	–
	TRH 20	5	4	5	4	4	4	4	+
70	Control	3	2	1	3	1	2	3	–
	TRH 20	3–4	4	5	5	4	4	5	+

TRH-induced behavioral arousal was estimated in intact developing rats. Behavioral observation was begun immediately after an IP injection of TRH (20 mg/kg) and continued for 60 min in rats at each stage with the exception of the observation period of 7 hr in 7-day-old rats. Each behavior was assessed by means of a rating scale as follows: Score 0, not observed; 1, very slight; 2, slight; 3, moderate; 4, strong; 5, marked. Straub tail response was expressed as: observed (+) or not observed (–). n=6–10 for each group.

Measurement of Locomotor Activity and Behavioral Observation

All behavioral studies were carried out between 10:00 and 17:00 in a quiet room at 23°C.

Each rat was placed into a plastic cage (24×18 cm) and walking (crawling), rearing, head, body and limb shaking, grooming (scratching), chewing, sniffing and licking (nibbling) were carefully observed and assessed by means of a rating scale as follows: Score 0, not observed; 1, very slight; 2, slight; 3, moderate; 4, strong; 5, marked. Straub tail response was also observed. Locomotor activity was measured with an ANIMEX activity meter (Type S, LKB Instrument, sensitivity, 40 μA). Recording of locomotor activity was for 5 min every 5 min for a period of 60 min in the 14-, 20-, 30- and 70-day-old rat. In 14- and 70-day-old animals, recording was also for 5 min at 180 min after a TRH injection. Since preliminary experimentation showed that the behavioral stimulant action of TRH (0.5 mg/kg to 20 mg/kg, SC) lasted for several hours in the 7-day-old animal pretreated with 6-OHDOPA, locomotor activity in this case was measured for a period of 7 hr.

In order to examine interactions between TRH and α-flupenthixol (FPT) and phenoxybenzamine (PBZ) in the 7-day-old rat, all animals were injected SC (0.1 ml/10 g of body weight) with TRH (0.5 mg/kg, 2 mg/kg or 20 mg/kg) at intervals of 30 min after an intraperitoneal (IP) injection of FPT (0.5 mg/kg or 1 mg/kg) or PBZ (5 mg/kg or 10 mg/kg). Locomotor activity of treated animals was measured for 5 min every 5 min between 1 hr and 3 hr after the IP injection of TRH and expressed as counts/60 min. TRH, FPT and PBZ were dissolved in saline.

The pA_2-values of the antagonists were calculated from the shift of the dose-response curve of TRH in the presence of the antagonists according to the equation $pA_2=pA_x+\log(x-1)$ proposed by Schild [32]. Student's t-test was used for statistical analysis.

Drugs

TRH tartrate monohydrate and α-flupenthixol hydrochloride were kindly donated by Takeda Chemical Industries, Ltd., and 6-hydroxydopa hydrochloride by Nippon-Roche Research Center. Phenoxybenzamine hydrochloride was purchased from Tokyo Kasei Ind. Co. Ltd.

RESULTS

Behavioral Effect of TRH in the Developing Rat

An IP injection of TRH (20 mg/kg) increased walking with sniffing, rearing, body shaking, grooming or scratching, chewing, licking and nibbling forelimbs in the adult rat between a few minutes and 50 to 60 min after the injection (Table 1). The Straub tail response was also produced by TRH (20 mg/kg, IP). The 7-, 14-, 20-and 30-day-old rats were responsive to TRH (20 mg/kg, IP), showing these behaviors (Table 1). In 7- and 14-day-old rats, TRH caused head, body and limb shaking, sniffing, licking and nibbling which were not observed in controls. The behavioral responsiveness to TRH in 20- and 30-day-old rats was almost similar to that in adult rats (Table 1). When locomotor activity was measured with an ANIMEX activity meter during 60 min after the injection, TRH increased locomotor activity in 14-, 20- and 30-day-old rats but not in 7-day-old ones (Fig. 1). However, TRH induced locomotor hyperactivity between 1.5 and 3.5 hr after the injection in 7-day-old rats but not in others (Fig. 1 and Fig. 2).

The Locomotor Stimulant Effect of TRH in 7-, 14-day-old and Adult Rats Pretreated with 6-OHDOPA

On Day 7, TRH induced behavioral changes such as crawling, grooming, scratching, body and limb shaking, chewing and licking or nibbling of forelimbs, which were marked in 6-OHDOPA-treated rats compared to controls

TABLE 2

TRH-INDUCED BEHAVIORAL AROUSAL AND ITS INHIBITION BY α-FLUPENTHIXOL AND PHENOXYBENZAMINE IN 6-OHDOPA-TREATED, 7-DAY-OLD RATS

Infant rats	Pretreatment (mg/kg)	Treatment (mg/kg)	Behavior observed: Crawling	Head, body & limb shaking	Grooming (scratching)	Chewing	Licking (nibbling)
Control	Saline	Saline	1	0	1	0	0
		TRH 0.5	1	1	2	1	2
		TRH 2	2	1	3	1	2
		TRH 20	3	3	4	4	4
6-OHDOPA	Saline	Saline	1	0	1	0	0
		TRH 0.5	3	2	3	2	2
		TRH 2	5	3	5	3	4
		TRH 20	4	4	5	4	5
	FPT 0.5	Saline	0–1	0	0–1	0	0
		TRH 0.5	1	2	1	1	1
		TRH 2	3	3	4	1	1
	PBZ 5	Saline	0–1	0	0–1	0	1
		TRH 0.5	1	1	1	1	2
		TRH 2	3	2	1	1	3

Control and 6-OHDOPA-treated, 7-day-old rats were subcutaneously injected with TRH (0.5 mg/kg, 2 mg/kg or 20 mg/kg). Behavioral observation was carried out between 1 hr and 3 hr after an injection of TRH and assessed by means of the rating scale as described in Table 1. In experiments concerning the interaction between TRH and α-flupenthixol (FPT) and phenoxybenzamine (PBZ), animals were subcutaneously injected with TRH (0.5 mg/kg or 2 mg/kg) at intervals of 30 min after an intraperitoneal injection of FPT (0.5 mg/kg) or PBZ (5 mg/kg). n=5 to 10 for each group.

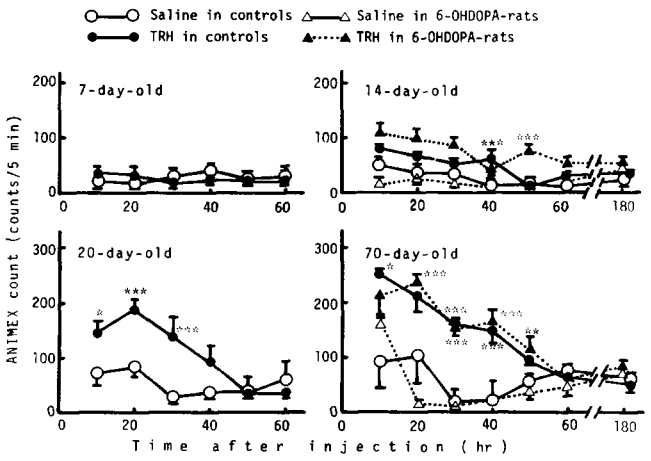

FIG. 1. The developmental change of TRH-induced locomotor stimulation in control and 6-OHDOPA-treated rats. Newborn rats were treated with 6-OHDOPA (75 mg/kg, SC) or the vehicle at 0, 2 and 4 days. Animals at each stage were placed singly into a plastic cage and their locomotor activity measured with an ANIMEX activity meter for 60 min after an IP injection of TRH (20 mg/kg) or saline. In case of 14-day-old and adult rats, locomotor activity was also measured at 180 min after a TRH injection. Locomotor activity is shown as the mean value ± SE for 6 to 8 determinations. Significance: ***$p<0.001$, **$p<0.01$, *$p<0.05$ vs saline.

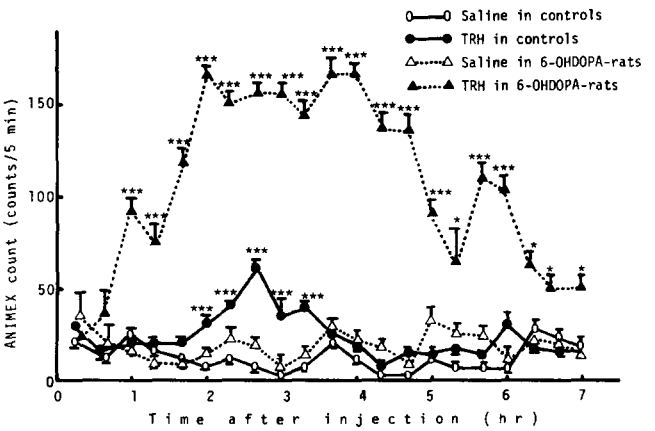

FIG. 2. Time course of TRH-induced locomotor stimulation in control and 6-OHDOPA-treated, 7-day-old rats. Newborn rats were treated with 6-OHDOPA (75 mg/kg, SC) or the vehicle at 0, 2 and 4 days. Animals at 7 days were placed singly into a plastic cage and their locomotor activity measured with an ANIMEX activity meter. Recording began 5 min after an IP injection of TRH (20 mg/kg). Locomotor activity was measured for 5 min at a time with a 5 min intermission between measurements. Locomotor activity is shown as the mean value ± SE for 8 to 10 determinations. Significance: ***$p<0.001$, *$p<0.05$ vs saline.

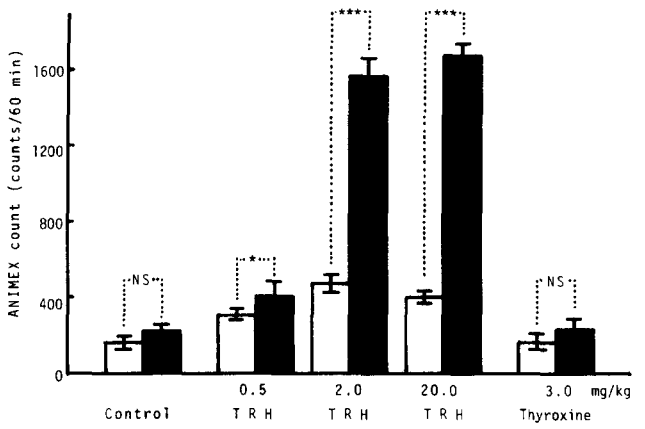

FIG. 3. The locomotor stimulant effects of TRH and thyroxine in control and 6-OHDOPA-treated, 7-day-old rats. Control or 6-OHDOPA-treated, 7-day-old rats were placed into a plastic cage and their locomotor activity measured as described in legend of Fig. 2. The measurements were accumulated for 12 consecutive observation periods during the 120 min session. Locomotor activity is shown as the mean value ± SE for 4 to 9 determinations. White column, controls; hatched column, 6-OHDOPA-treated. Significance: ***$p<0.001$, **$p<0.01$, *$p<0.05$ vs controls NS, not significant.

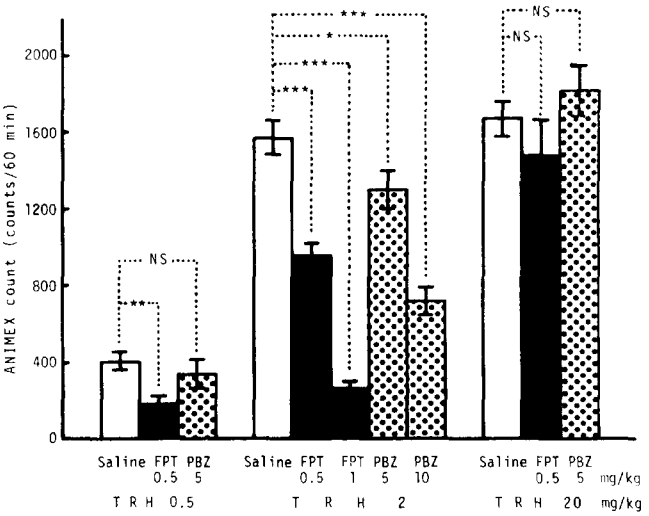

FIG. 4. The effects of α-flupenthixol (FPT) and phenoxybenzamine (PBZ) on TRH-induced locomotor stimulation in 6-OHDOPA-treated, 7-day-old rats. Control and 6-OHDOPA-treated animals received an IP injection of FPT (0.5 mg/kg or 1 mg/kg) or PBZ (5 mg/kg or 10 mg/kg) before an IP injection of TRH (0.5 mg/kg, 2 mg/kg or 20 mg/kg). The measurements were accumulated for 12 consecutive observation periods during the 120 min session. Locomotor activity is shown as the mean value ± SE for 5 to 10 determinations. Significance: ***$p<0.001$, **$p<0.01$, *$p<0.05$ vs controls. NS, not significant.

(Table 2). Neonatal treatment with 6-OHDOPA resulted in potentiation in TRH-induced locomotor hyperactivity which lasted for apprxoimately 7 hr in 7-day-old rat (Fig. 2) but not in 14-day-old and adult animals during 3 hr after TRH injection (Fig. 1). In 6-OHDOPA-treated, 7-day-old animals, the stimulant effect of TRH lasted for approximately 7 hr and was dose-dependent in a dose range of 0.5 mg/kg to 20 mg/kg

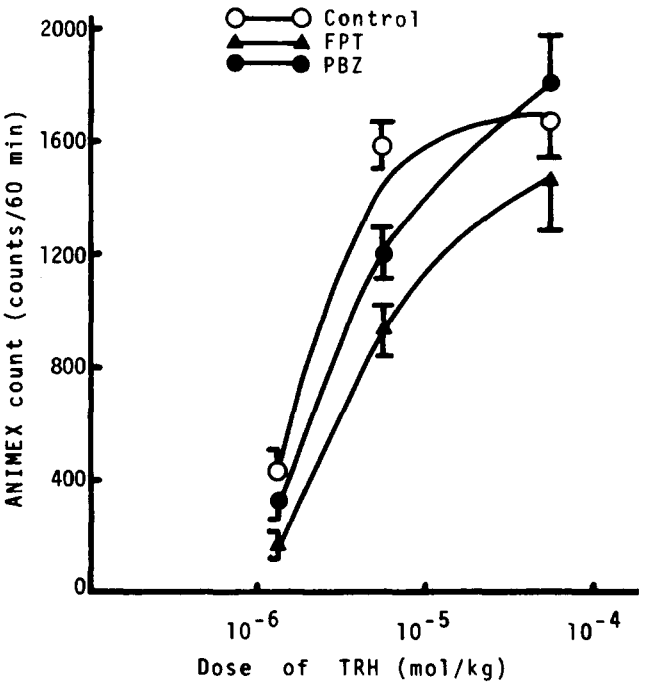

FIG. 5. The effects of α-flupenthixol (FPT) and phenoxybenzamine (PBZ) on the dose-response curve for TRH-induced locomotor stimulation. Seven-day-old rats neonatally SC treated with 6-OHDOPA were IP injected with TRH in a dose range of 0.5 mg/kg (1.38×10^{-6} mol/kg) to 20 mg/kg (5.52×10^{-5} mol/kg) after 30 min of an IP injection of FPT (0.5 mg/kg=1.06×10^{-6} mol/kg) or PBZ (5 mg/kg=1.47×10^{-5} mol/kg). The results are expressed as the mean value ± SE for locomotor activity (counts/60 min). pA_2-values were calculated from the shift of the dose-response curve of TRH in the presence of antagonists according to the equation $pA_2=pA_x+\log(x-1)$ proposed by Schild [32]. The pA_2-values were determined as 5.9 (FPT) and 4.4 (PBZ).

(Fig. 3). On the other hand, thyroxine (3 mg/kg, IP) produced neither behavioral changes nor locomotor stimulation in 6-OHDOPA-treated rats as well as controls on Day 7 (Fig. 3).

Influences of α-Flupenthixol and Phenoxybenzamine on TRH-induced Behavioral Changes and Locomotor Stimulation in 7-day-old Rats Pretreated with 6-OHDOPA

A preceding IP injection of FPT (0.5 mg/kg), a strong DA receptor blocker, significantly reduced locomotor stimulation induced by TRH (0.5 mg/kg and 2 mg/kg) in 6-OHDOPA-treated, 7-day-old animals but not that induced by TRH at 20 mg/kg (Fig. 4). PBZ (5 mg/kg and 10 mg/kg), an α-adrenoceptor blocker, affected locomotor stimulation by TRH (2 mg/kg) but the antagonistic potency was lower than that of TRH (Fig. 4). Figure 5 shows the effects of increasing concentrations of TRH on locomotor stimulation in 6-OHDOPA-treated, 7-day-old rats in combination with FPT (0.5 mg/kg=1.06×10^{-6} mol/kg) or PBZ (5 mg/kg=1.47 mol/kg). From these results, pA_2-values were calculated as follows: 5.9 (FPT); 4.4 (PBZ). Both FPT (0.5 mg/kg) and PBZ (5 mg/kg) attenuated TRH-induced crawling, grooming, chewing and licking in 6-OHDOPA-treated infant rats (Table 2).

DISCUSSION

TRH produced hyperactivity associated with body shaking and head and limb movement in adult rats. TRH-induced behavioral changes in 7-day-old rats were protypic but similar to those in adult ones. Responsiveness in 14- and 20-day-old rats was almost the same as that in the adult, suggesting that the physiological mechanism involving TRH-induced hyperactivity reaches maturity at approximately 2 and 3 weeks after birth in the rat. The TRH-induced behavioral arousal seems to be expressed as locomotor stimulation which can be measured by an ANIMEX activity meter. As suggested by Costall *et al.* [4], counts reflect the general state of excitability of the rat rather than a true expression of locomotor activity. Neonatal treatment with 6-OHDOPA did not affect TRH-induced locomotor hyperactivity in both 14-day-old and adult rats. This is consistent with the results of Miyamoto *et al.* [15] and Vogel *et al.* [33] in which they have reported that a preceding intraacumbens or intracisternal injection of 6-OHDA did not affect TRH-induced hyperactivity in adult animals. However, Heal and Green [6] demonstrated that the locomotor stimulant action of TRH is abolished by the intracisternal administration of 6-OHDA and suggested that locomotor stimulation by TRH is due to DA release in the nucleus accumbens.

In 6-OHDOPA-treated, 7-day-old rats, locomotor hyperactivity induced by TRH was much higher in degree and much longer in duration than in controls. Since an injection of thyroxine (3 mg/kg, IP) elicited no behavioral arousal in infant rats treated with 6-OHDOPA, it seems possible that peripherally injected TRH is not acting by release of thyrotropine and thyroxine. No behavioral effects of thyrotropine and thyroxine have been demonstrated in the adult rat [6] and fowl [16]. Although the phenomenon that TRH-induced behavioral arousal was markedly potentiated by treatment with 6-OHDOPA in only infant rats is not clearly explained at the moment, it is likely to be due to the following: (1) difference in pharmacokinetic factors, e.g., distribution and inactivation of TRH between 7-day-old animals and ones older than 7 days; (2) receptor hypersensitivity to TRH by treatment with 6-OHDOPA. Concerning the former possibility, the enzymology of TRH is not yet well worked out, although 10% of the inactivating enzyme is localized in synaptosomal fractions in the brain [8]. Pharmacokinetic factors responsible for TRH inactivation perhaps are lower in infant animals than in adult ones. Neonatal treatment with 6-OHDOPA seems to cause hypersensitivity to TRH in the CNS on Day 7, since maximal response to TRH in locomotor hyperactivity markedly increased in 6-OHDOPA-treated infants compared to controls.

Behavioral arousal and locomotor hyperactivity induced by TRH in 6-OHDOPA-treated infant rats was reduced by a systemic injection of FPT (0.5 mg/kg). PBZ (5 mg/kg) also was shown to attenuate behavioral arousal and locomotor hyperactivity induced TRH. The pA_2-values of FPT (5.9) and PBZ (4.4) suggest that DA receptors rather than α-adrenoceptors are involved in 6-OHDOPA-induced potentiation in the behavioral stimulant action of TRH, although the involvement of noradrenergic neurons cannot be ruled out. We found that central DA receptors as well as α-adrenoceptors become functionally sensitive at least by 7 days after birth in the rat [18,21]. A high K^+-induced, Ca^{2+}-dependent release of (^{3}H) NA and (^{3}H) DA from brain slices as well as the high affinity uptake system of (^{3}H)NA and (^{3}H)DA exists in the brain of the newborn rat [5, 10, 20, 23], although the content and biosynthetic enzymes for catecholamine are only 10–20% of adult [17,20]. Specific binding of (^{3}H)-haloperidol in the striatum has been reported to be between 10 and 15% of adult levels at birth [27]. It seems probable that central DA receptors which are not fully sensitive, even if sensitive [26], to DA agonists and antagonists on Day 7 reach functional maturity by 20 days of the postnatal life in the rat [24]. With regard to the interaction of TRH with DA receptors, Burt and Snyder [2] demonstrated that DA at 10 μM does not inhibit the high affinity specific binding of (^{3}H)TRH in synaptic membrane fractions of the cerebral cortex in adult rats. By contrast, TRH in doses of 10^{-5} M to 10^{-4} M caused (^{14}C)DA release from slices of the rat nucleus accumbens septi [15]. Therefore, TRH may not directly act on central DA receptors but induce DA release in the infant. We found that neonatal 6-OHDOPA treatment reduced cortical DA content as well as NA content and that increased the specific binding (^{3}H)spiroperidol binding in the striatum in developing rats ([22], Nomura and Oki, unpublished observation). Thus, 6-OHDOPA perhaps induces the degeneration of DA terminals, subsequently resulting in supersensitivity to DA receptors in the striatum and/or the nucleus accumbens. The histochemical finding [25] that DA fluorescence in the nucleus accumbens appears at an earlier postnatal age than that in the striatum may suggest more important relationship of nucleus accumbens DA system to TRH action. On the other hand, central α-adrenoceptors also should be involved in TRH effect, not just DA receptors. The fact that marked locomotor hyperactivity due to TRH in 6-OHDOPA-treated animals was not observed in 14-day-old ones suggests that physiological and pharmacological factors rapidly change during 1 and 2 weeks after birth.

Further works are required to clarify the details of the mechanism (e.g., the involvement of the central DA and/or NA system) of the behavioral stimulant action of TRH as well as its pharmacokinetics in the developing rat.

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REFERENCES

1. Agarwal, R. A., R. B. Rastogi and R. L. Singhal. Enhancement of locomotor activity and catecholamine and 5-hydroxytryptamine metabolism by thyrotropin releasing hormone. *Neuroendocrinology* **23:** 236–247, 1977.

2. Burt, D. R. and S. H. Snyder. Thyrotropin releasing hormone (TRH): Apparent receptor binding in rat brain membranes. *Brain Res.* **93:** 309–328, 1975.

3. Campbell, B. A., L. D. Lytle and H. C. Fibiger. Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* **166:** 635–636, 1969.
4. Costall, B., S. -C. G. Hui, G. Metcalf and R. J. Naylor. A Study of the changes in motor behaviour caused by TRH on intracerebral injection. *Eur. J. Pharmac.* **53:** 143–150, 1979.
5. Coyle, J. T. and J. Axelrod. Development of the uptake and storage of L-(^{3}H)norepinephrine in the rat brain. *J. Neurochem.* **18:** 2061–2075, 1971.
6. Heal, D. J. and A. R. Green. Administration of thyrotropin releasing hormone (TRH) to rats releases dopamine in n. accumbens but not n. caudatus. *Neuropharmacology* **18:** 23–31, 1979.
7. Hökfelt, T., K. Fuxe, O. Johansson, S. Jeffcoate and N. White. Distribution of thyrotropin-releasing hormone (TRH) in the central nervous system as revealed with immunohistochemistry. *Eur. J. Pharmac.* **34:** 389–392, 1975.
8. Joseph-Bavo, P., C. Loudes, J. L. Charli and C. Kordon. Subcellular distribution of brain peptidases degrading lutenizing hormone releasing hormone (LHRH) and thyrotropin-releasing hormone (TRH). *Brain Res.* **166:** 321–329, 1979.
9. Kellogg, C. and P. Lundborg. Ontogenic variations in responses to L-DOPA and monoamine receptor-stimulating agents. *Psychopharmacologia* **23:** 187–200, 1972.
10. Kirksey, D. F. and T. A. Slotkin. Concomitant development of (^{3}H)-dopamine and (^{3}H)-5-hydroxytryptamine uptake systems in rat brain regions. *Br. J. Pharmac.* **67:** 387–396, 1979.
11. Kulig, B. M. The effects of thyrotropin-releasing hormone on the behaviour of the rats pretreated with α-methyl tyrosine. *Neuropharmacology* **14:** 489–492, 1975.
12. Lanier, L. P. and R. L. Isaacson. Early developmental changes in the locomotor response to amphetamine and their relation to hippocampal function. *Brain Res.* **126:** 567–575, 1977.
13. Miller, R. and B. Sahakian. Differential effects of neuroleptic drugs on amphetamine-induced stimulation of locomotor activity in 11-day-old and adult rats. *Brain Res.* **81:** 387–392, 1974.
14. Miyamoto, M. and Y. Nagawa. Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats. *Eur. J. Pharmac.* **44:** 143–152, 1977.
15. Miyamoto, M., S. Narumi, Y. Nagai, T. Shima and Y. Nagawa. Thyrotropin releasing hormone: Hyperactivity and mesolimbic dopamine system in rats. *Jap. J. Pharmac.* **29:** 335–347, 1979.
16. Nistico, G., D. Rotiori, A. De Sarro and J. D. Stephenson. Behavioral electrocortical and body temperature effects after intracerebral infusion of TRH in fowls. *Eur. J. Pharmac.* **50:** 253–260, 1978.
17. Nomura, Y., F. Naitoh and T. Segawa. Regional change in monoamine content and uptake of the rat brain during postnatal development. *Brain Res.* **101:** 305–315, 1976.
18. Nomura, Y. and T. Segawa. Apomorphine-induced locomotor stimulation in the developing rat treated with 6-hydroxydopa. *Eur. J. Pharmac.* **50:** 153–156, 1978.
19. Nomura, Y. and T. Segawa. Muscarinic hyposensitivity in the developing rat pretreated with 6-hydroxydopa. *Eur. J. Pharmac.* **50:** 431–435, 1978.
20. Nomura, Y., Y. Tanaka and T. Segawa. Influences of sodium, catecholamine and tricyclic antidepressant drug on L-(^{3}H)norepinephrine uptake into synaptosomal fractions of developing rat brain. *Jap. J. Pharmac.* **28:** 501–504, 1978.
21. Nomura, Y. and T. Segawa. The effect of α-adrenoceptor antagonists and metiamide on clonidine-induced locomotor stimulation in the infant rat. *Br. J. Pharmac.* **66:** 531–535, 1979.
22. Nomura, Y. and T. Segawa. Striatal dopamine content reduced in developing rats treated with 6-hydroxydopa. *Jap. J. Pharmac.* **29:** 306–309, 1979.
23. Nomura, Y., I. Yotsumoto and T. Segawa. High potassium-induced, calcium-dependent monoamine release from brain slices of the newborn rat. *Jap. J. Pharmac.* **29:** 847–854, 1979.
24. Nomura, Y., K. Oki and T. Segawa. Apomorphine- and haloperidol-induced change in 3,4-dihydroxyphenylacetic acid content in the mesolimbic-striatum in the developing rat. *J. Pharmacobio-Dyn.* **3:** 112–117, 1980.
25. Olson, L., A. Seiger and K. Fuxe. Heterogeneity of striatal and limbic DA fluorescent islands in the developing and adult rat. *Brain Res.* **44:** 283–288, 1972.
26. Ormond, D. L. and C. V. Hartesveldt. Functional development of dopamine receptors in the rat forebrain. *Pharmac. Biochem. Behav.* **10:** 855–860, 1979.
27. Pardo, J. V., I. Creese, D. R. Burt and S. H. Snyder. Ontogenesis of dopamine receptor binding in the corpus striatum of the rat. *Brain Res.* **125:** 376–382, 1977.
28. Plotonikoff, N. P., A. J. Prange, G. R. Breese, M. S. Anderson and L. C. Wilson. Enhancement of DOPA activity by a hypothalamic hormone. *Science* **178:** 417–418, 1972.
29. Plotonikoff, N. P. Thyrotropin-releasing hormone: Enhancement of DOPA activity in thyroidectomized rats. *Life Sci.* **14:** 1271–1278, 1974.
30. Porcher, W. and A. Heller. Regional development of catecholamine biosynthesis in rat brain. *J. Neurochem.* **19:** 1917–1930, 1972.
31. Reinstein, D. K. and R. L. Isaacson. Clonidine sensitivity in the developing rat. *Brain Res.* **135:** 378–382, 1977.
32. Schild, H. O. pA_x and competitive drug antagonism. *Br. J. Pharmac.* **4:** 98–100, 1949.
33. Vogel, R. A., B. R. Cooper, T. S. Barlow, A. J. Prange, Jr., R. A. Mueller and G. R. Breese. Effects of thyrotropin-releasing hormone on locomotor activity, operant performance and ingestive behaviour. *J. Pharmac. exp. Ther.* **202:** 161–168, 1979.
34. Winkur, A. and R. D. Utiger. Regional distribution of thyrotropin-releasing hormone in rat brain. *Science* **185:** 265–267, 1974.