INFLUENCE OF NEONATAL TREATMENT WITH 6-HYDROXYDOPA ON CLONIDINE-INDUCED LOCOMOTOR STIMULATION IN THE INFANT RAT

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Several studies have shown that clonidine produces locomotor stimulation in the infant rats aged 1 to 14 days, but causes hypoactivity in the adult animals (1–4). The fact that clonidine-induced locomotor stimulation is reduced by a preceding intraperitoneal injection of several α -adrenoceptor antagonists, suggests that clonidine induces locomotor stimulation by activating α -adrenoceptors in the central nervous system of the infant rat (5). In the adult rats, α -adrenoceptors which mediate clonidine-induced sedation more resemble the peripheral presynaptic α -adrenoceptors than the postsynaptic ones (6). Furthermore, it has been suggested that clonidine predominantly activates presynaptic α -adrenoceptors and thereby induces sedation in the adult rats (7), while Spyraiki and Fibiger (8) have suggested that the clonidine-induced sedation is mediated by postsynaptic α_2 -receptors in adult rats. In addition to this controversial idea, it is not clear how reversal in the locomotor effect of clonidine occurs during postnatal life in rats.

To gain insight into the mechanism of the locomotor effect of clonidine in developing rats, the author investigated the effects of neonatal treatment with 6-hydroxydopa (6-OHDOPA) on clonidine-induced behavioral changes in 7-day-old rats.

Seven-day-old Wistar rats of either sex were used. After birth, all litters were culled to 10–12 litters/mother. 6-OHDOPA (75 mg/

kg), dissolved in saline solution containing 0.001 N HCl, or vehicle was first injected subcutaneously (s.c.) into rats within several hr after birth, followed by s.c. injection at 2 and 4 days. Treated pups were kept with a mother under daylight conditions at 23°C. Locomotor activity of each rat was measured in a plastic cage (24×18 cm) with an ANIMEX activity meter (Type S, LKB Instrument, sensitivity: 40 μ A) by the method previously reported (5). The recording began 10 min after the s.c. injection of clonidine (2.0×10^{-7} mol/kg or 7.8×10^{-7} mol/kg). Locomotor activity was measured for 10 min every 20 min for a period of 90 min and expressed as counts/50 min. Other behaviors of wall climbing, forward crawling, body tremor and single clonic jerk were carefully observed and recorded during the measurement of locomotor activity. Clonidine hydrochloride was generously provided by C.H. Boehringer Ingelheim Ltd.; 6-OHDOPA hydrochloride was kindly donated by Nippon-Roche Research Center.

A s.c. injection of clonidine (2.0×10^{-7} mol/kg or 7.8×10^{-7} mol/kg) induced intense forward crawling, wall climbing, head raising and body tremor initiated by single clonic jerk and resulted in a significant increase in locomotor activity (Fig. 1). Neonatal treatment with 6-OHDOPA attenuated clonidine-induced wall climbing and forward crawling and significantly reduced clonidine-induced locomotor stimulation (Table 1). However,

Fig. 1. The effect of neonatal treatment with 6-hydroxydopa on clonidine-induced locomotor stimulation and behavioral changes in the infant rats. 6-Hydroxydopa (75 mg/kg) was injected s.c. into the newborn rats on days 0, 2 and 4. Animals were s.c. injected clonidine (2.0×10^{-7} mol/kg or 7.8×10^{-7} mol/kg). Locomotor activity was measured, and behavior was observed. The results are the mean effects on locomotor activity (counts/50 min); vertical bars show S.E. of the mean values. Hatched columns are 6-hydroxydopa-treated groups, and open columns are vehicle-treated groups. * $P < 0.05$ and ** $P < 0.01$ vs. vehicle treatment. NS: not significant vs. vehicle treatment.

Table 1. Influence of clonidine on general behaviors in 7-day-old rats pretreated with 6-hydroxydopa

Treatment (mol/kg)	Pretreatment	The number of animals	General behaviors			
			Wall climbing	Forward crawling	Body tremor	Clonic jerk
Saline	Vehicle	13	—	±	—	—
	6-OHDOPA	11	—	—	—	—
Clonidine 2.0×10^{-7}	Vehicle	8	++	++	+	+
	6-OHDOPA	11	±	+	+	++
Clonidine 7.8×10^{-7}	Vehicle	10	+++	+++	+	+
	6-OHDOPA	13	+	++	++	++

6-Hydroxydopa, 75 mg/kg, was injected s.c. into neonatal rats on days 0, 2 and 4. Animals were s.c. injected with clonidine (2×10^{-7} or 7.8×10^{-7} mol/kg). Wall climbing, forward crawling, body tremor and clonic jerk were observed for a period of 90 min after an injection of clonidine. Behavioral responses were graded as: ++++, marked; ++, moderate; +, slight; ±, slight or no change; —, no change.

body tremor and single clonic jerk induced by clonidine were more frequent in 6-OHDOPA-treated infant rats than in control ones (Table 1).

In comparison to clonidine-induced hypoactivity in adults (4), the drug caused hyperactivity in 7-day-old rats. The different locomotor effects of clonidine in developing animals may reflect the regional variation of developing stage when noradrenaline (NA) neurons innervate and functionally mature (9). The results presented here also show that the neonatal treatment with 6-OHDOPA reduced clonidine-induced locomotor hyperactivity such as wall climbing and forward crawling, but slightly increased clonidine-induced body tremor and single jerk. S.c. injection of 6-hydroxydopamine (6-OHDA) (100 mg/kg, days 1, 3 and 5 after birth) caused a significant reduction in telodiencephalon NA content in 7-day-old rats (10). We found that 10^{-7} M clonidine significantly decreased 20 mM K^{+} -induced L-[3H]NA release from cerebral cortical slices in the 7-day-old rats (11). Therefore, it is presumable that neonatal treatment with 6-OHDOPA degenerates presynaptic terminals of NA neurons in which clonidine acts and subsequently inhibits NA release. Thus the receptors which mediate clonidine-induced locomotor hyperactivity could be presynaptic α_2 -adrenoceptors in the central nervous system of the infant animals. This is consistent with our previous observations that yohimbine and piperoxane (preferential blockers of α_2 -adrenoceptors) have high pA_2 -values than phenoxybenzamine (a blocker of α_1 -adrenoceptor) against clonidine-induced hyperactivity in infant rats (5). On the other hand, the increasing effect of clonidine on body tremor and single jerk was enhanced by neonatal 6-OHDOPA, indicating that in the infant rats, central α_2 -adrenoceptors mediating these behaviors may largely be located postsynaptically since up-regulation

in postsynaptic α_2 -receptors seems to occur on day 7 following 6-OHDOPA treatment on days 0, 2 and 4. The brain stem NA content is elevated following neonatal treatment with 6-OHDOPA (12, 13) in the 2-week-old rats, which is explained by collateral accumulation, in contrast to reduction in telodiencephalon NA content by 6-OHDA (10). It is not ruled out that the modification in the behavioral effects of clonidine by 6-OHDOPA treatment may originate from 6-OHDOPA-induced changes of regional innervation of NA neurons during early development. Although 6-OHDOPA attenuated clonidine-induced locomotor hyperactivity such as wall climbing and forward crawling, 6-OHDOPA rather enhanced clonidine-induced body tremor and single jerk. Thus the two kinds of behaviors elicited by clonidine seem to be due to its stimulation of different sites (receptors and/or brain regions) in the infant animals.

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