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Age-related changes in the central catecholaminergic function and its interaction with methamphetamine during postnatal life in the rat

YASUYUKI NOMURA*, IKUKO YOTSUMOTO, KEIKO OKI, *Department of Pharmacology, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan*

The onset of the behavioural action of amphetamine varies according to the different behavioural components examined and the source of the reports (Campbell et al 1969; Lal & Sourkas 1973; Miller & Sahakian 1974; White & Tapp 1977). Although the striatal dopamine (DA) system has been suggested as being responsible for amphetamine hypermotility (Thornburg & Moore 1973), the central noradrenaline (NA) system seems to be involved in amphetamine-hyperactivity but the DA system in its stereotypy (Snyder et al 1979). In contrast, locomotor activation and stereotypy induced by amphetamine are mediated by the mesolimbic-mesocortical (A_{10} group) and nigrostriatal (A group) DA systems, respectively (Kelly et al 1975; Costall et al 1977; Iversen & Koob 1977).

We wished to examine the ontogenesis of the synaptic function of the central catecholaminergic system and the biochemical mechanism of locomotor stimulating action of methamphetamine.

Albino rats (Wistar) of either sex were used. After birth all litters were culled to 10–12 pups/mother. Each rat was placed in a plastic cage (24 × 18 cm) and locomotor activity was measured with an ANIMEX activity meter (Typs S, LKB Instrument, sensitivity 40 μA) by the method of Nomura & Segawa (1979). Locomotor activity was measured for 10 min with a 10 min interval for 90 min and expressed as the sum of the five measurements as counts/50 min.

Table 1. The effect of methamphetamine on the high affinity uptake of (−)-[^{3}H]NA and [^{3}H]DA in 7- and 70-day-old rats. P_2 fractions were prepared from the diencephalon and striatum in both 7- and 70-day-old rats and suspended in Krebs-Ringer solution (pH 7·4). Aliquots of 1·6 ml were incubated with 0·2 ml of 4 concentrations of methamphetamine and 0·2 ml of radioactive NA (diencephalon) or DA (striatum) (final concentration, $1·0 \times 10^{-7}$ M) in triplicate, incubation was carried out for 10 min at 37 °C. IC50 values were derived from log-probit plots of per cent inhibition and expressed as means ± standard error of three or four independent determinations. The control uptakes (pmol mg^{-1} protein/10 min) were (−)-[^{3}H]NA, 1·19 ± 0·1/(7 days) and 1·30 ± 0·11 (70 days), [^{3}H]DA, 13·17 ± 1·79 (7 days) and 18·18 ± 2·91 (70 days). Significance, $^*P < 0·05$ vs 7-day-old.

Developmental stage	IC50 of methamphetamine	
	(−)-[^{3}H]NA uptake	[^{3}H]DA uptake
7-day-old	$(3·57 \pm 0·59) \times 10^{-6}$	$(1·11 \pm 0·07) \times 10^{-6}$
70-day-old	$(1·82 \pm 0·12) \times 10^{-6*}$	$(1·20 \pm 0·17) \times 10^{-6}$

* Correspondence.

The uptake experiment of [^{3}H]DA and (—)-[^{3}H]NA into P_2 fractions from the striatum and diencephalon was according to Nomura et al (1976). Preliminary experiments showed that the uptakes of [^{3}H]DA and (—)-[^{3}H]NA gradually increased with incubation time, to reach a plateau at approximately 15 and 30 min respectively.

The release experiment was performed by the modified method of Nomura et al (1979). Slices of striatum and diencephalon from rats of various postnatal ages were suspended in the Krebs-Ringer solution containing pheniprazine 2×10^{-5} M and 0·2% ascorbic acid. Samples (1·5 ml) of each suspension were pre-incubated for 20 min at 37 °C and then [^{3}H]DA (15 μl) (final concn $1·0 \times 10^{-7}$ M) or (—)-[^{3}H]NA ($1·0 \times 10^{-7}$ M) was added and the suspensions further incubated at 37 °C for 20 min. The slices preloaded radioactive amines were placed on a paper disc (Toyo filter No. 2) held in the filter holder and perfused with freshly prepared Krebs-Ringer solution (pH 7·4, 37 °C) at a rate of 0·25 ml min^{-1}. Twenty-two min after the start of the perfusion, methamphetamine 10^{-6} or 10^{-5} M was perfused for 20 min. The perfusate was collected into tubes (0·5 ml/tube) and the radioactivity was determined. Preliminary experiments on analysis of radioactivity in the perfusates by a high pressure liquid chromatography (Waters, TSk-GEL LS-410 ODS column) and a scintillation spectrometer (Packard Model 3325) showed that more than 78–80% radioactivity was unmetabolized amines.

Statistical differences were analysed by Student's *t*-test.

[^{3}H]DA hydrochloride (5 Ci mmol^{-1}) and (—)-[^{3}H]NA (5 Ci mmol^{-1}) were purchased from Radiochemical Centre, Amersham and methamphetamine hydrochloride from Dainippon Pharmaceutical Co Ltd, Osaka, Japan. All drugs were dissolved in 0·9% NaCl for use.

Methamphetamine 0·5, 1·0 and 2·0 mg kg^{-1} produced forward crawling and a dose-dependent increase in locomotor activity in 1-day-old rats (Fig. 1). Methamphetamine (1·0 mg kg^{-1})-induced hypermotility reached a peak on day 20 which declined by days 35 ($P < 0·01$) and 70 ($P < 0·05$). Methamphetamine 0·5 to 2·0 mg kg^{-1} caused stereotypy by day 14 and at 10 mg kg^{-1} it caused prototype sniffing in 1-day-old pups.

The inhibitory potency of methamphetamine on the synaptosomal uptake of [^{3}H]DA (striatum) and

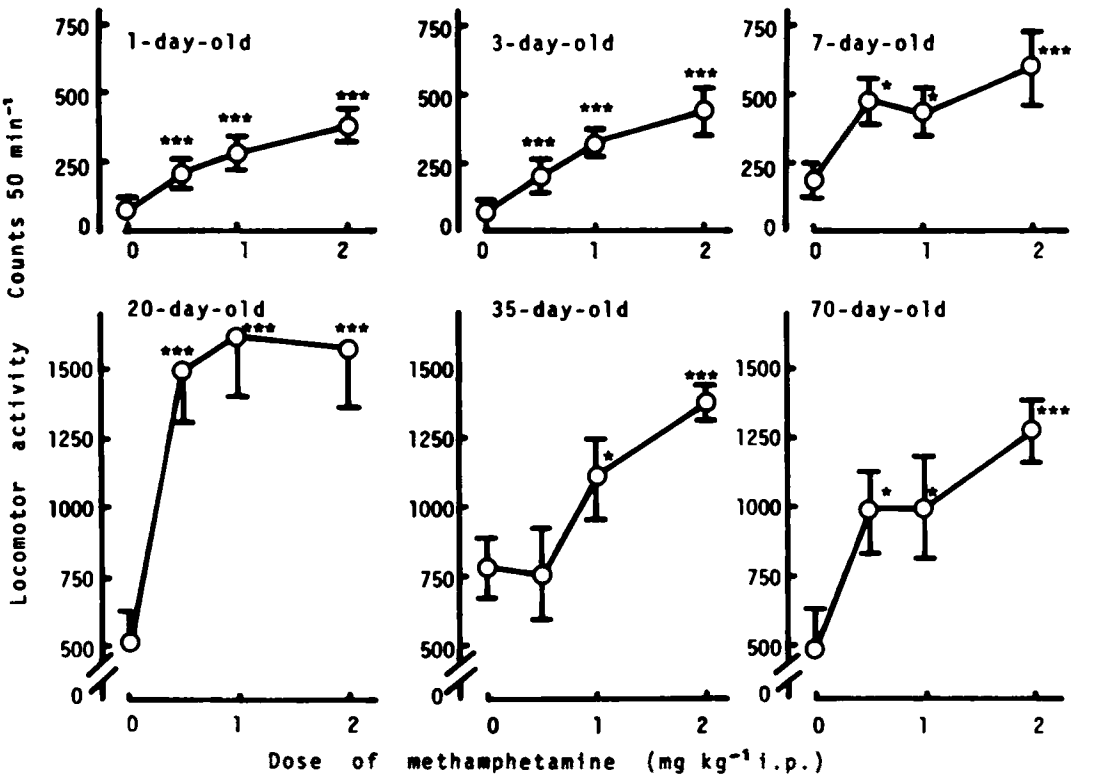

FIG. 1. Developmental changes in methamphetamine-induced locomotor stimulation in the rat. Animals at each stage were placed singly into a plastic cage and their locomotor activity measured with an ANIMEX activity meter as described in the text. Locomotor activity is shown as mean ± standard error for 8 to 16 determinations. Significance, $***P < 0.001$, $*P < 0.05$ vs control.

(—)-[^{3}H]NA (diencephalon) was examined on day 7 and day 70 and expressed as the 50% inhibitory concentration (IC50) of the initial, high affinity uptake of both radioactive amines (Table 1). Methamphetamine was 3·2 fold more effective in inhibiting the [^{3}H]DA uptake than in (—)-[^{3}H]NA on day 7 and 1·5 fold on day 70. The [^{3}H]DA uptake was inhibited by methamphetamine in infants to the same extent as that in adults. In contrast, the inhibitory potency of methamphetamine on (—)-[^{3}H]NA uptake was significantly ($P < 0.05$) lower on day 7 than that on day 70.

Methamphetamine 10^{-6} and 10^{-5} M caused release 1·1 and 5·9% of total radioactivity in 1-day-old animals and the effect rapidly increased until day 7 (Table 2). DA release by methamphetamine on day 7 was of the same order of magnitude as that in adults. Only a little (—)-[^{3}H]NA release was evoked from slices of the neocortex and diencephalon by perfusion with methamphetamine 10^{-6} and 10^{-5} M at both 7 and 70 days (data not shown).

Methamphetamine increased locomotor activity on day 1, suggesting that synapses in the central DA and/or NA system already pay a physiological role at birth. The fact that methamphetamine hyperactivity was significantly decreased in 35- and 70-day-old compared with that in 20-day-old rats may be explained by: (i) the maturation of the blood-brain barrier, less methamphetamine enters the brain with increasing age, (ii) drug metabolism changes with age, and (iii) an inhibitory influence develops e.g. from the hippocampus (Lanier & Isaacson 1974) that overrides the excitatory effects of methamphetamine on the still-maturing catecholamine systems. Methamphetamine more effectively modified the DA uptake and storage systems than those for NA at 7 days, suggesting that action on the central DA transport and storage systems, rather than that on the NA systems, underlies methamphetamine-induced hypermotility in the newborn rat. Biochemical findings such as low but significant levels of DA (Nomura et al 1976), tyrosine hydroxylase (McGeer et al 1971) and of specific [^{3}H]spiperone binding (Nomura et al unpublished) in 1-day-old rats suggest the existence of functional synapses in the striatal DA system at birth. Since pre- and postsynaptic activities are also functional in the central NA system at birth (Kellogg & Lundborg 1972; Nomura & Segawa 1979), a facilitation by methamphetamine of the NA system and the induction of hyperactivity in developing rats cannot be ruled out.

Table 2. Development change in methamphetamine-induced [^{3}H]DA release from striatal slices. Striatal slices preloaded with [^{3}H]DA were placed on filter paper in a superfusion chamber and continuously superfused with Krebs-Ringer solution. The amount of methamphetamine-induced release of [^{3}H]DA was calculated by subtracting the estimated amount of [^{3}H]DA (amount in Krebs-Ringer solution) from the total [^{3}H]DA released during perfusion of the drug. Radioactivity released by methamphetamine is expressed as a percentage of total radioactivity recovered (total fractions plus the filter disc). The uptake capacities were 0·43 (1-day-old), 0·87 (7-day-old) and 1·03 pmol mg^{-1} wet tissue weight/20 min (70-day-old). The values in Table are the mean ± standard error with the number of experiments in parentheses.

	[^{3}H]DA release (Per cent of total radioactivity)			
Methamphetamine	1-day-old	3-day-old	7-day-old	70-day-old
10^{-5} M	1·1 ± 0·1 (4)	2·0 ± 0·5 (10)	6·0 ± 0·5 (5)	5·6 ± 0·4 (4)
10^{-4} M	5·9 ± 0·6 (4)	6·8 ± 0·7 (4)	13·9 ± 1·3 (6)	14·3 ± 1·0 (4)

In contrast, strong isolated areas of DA fluorescence in the nucleus accumbens appear at an earlier postnatal age than diffuse DA fluorescence in the striatum (Olson & Seiger 1972).

Methamphetamine appears to cause hyperactivity in 1-day-old rats by stimulation of the central DA transmission.

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