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BRIEF COMMUNICATION

Neurochemical Consequences Following Injection of the Substance P Analogue, DiMe-C7, Into the Rat Ventral Tegmental Area

J. M. BARNES, N. M. BARNES, B. COSTALL, A. J. COX, A. M. DOMENEY, M. E. KELLY AND R. J. NAYLOR

Postgraduate Studies in Pharmacology, The School of Pharmacy University of Bradford, Bradford, West Yorkshire, BD7 1DP, UK

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BARNES, J. M., N. M. BARNES, B. COSTALL, A. J. COX, A. M. DOMENEY, M. E. KELLY AND R. J. NAYLOR. *Neurochemical consequences following injection of the substance P analogue, DiMe-C7, into the rat ventral tegmental area.* PHARMACOL BIOCHEM BEHAV **37**(4) 839–841, 1990.—The effect on forebrain catecholamine- and indoleamine-related neurochemical levels was investigated following stimulation of the rat ventral tegmental area with the substance P analogue, DiMe-C7. DiMe-C7 (6.0 μg) induced a marked hyperactivity in rats with maximal response between 15 and 30 min following the injection. Fifteen min following the DiMe-C7 injection levels of dopamine and/or its metabolites (3,4-dihydroxyphenylacetic acid, homovanillic acid) were significantly increased in the nucleus accumbens, amygdala, entorhinal cortex and striatum relative to vehicle-injected animals. Although the increase in dopamine metabolism in the nucleus accumbens is consistent with the behavioural hyperactivity, it is concluded that other forebrain nuclei may also be involved in the mediation of the hyperactivity response.

Substance P DiMe-C7 Ventral tegmental area

IT is well established that injection of the undecapeptide putative neurotransmitter, substance P, into the rat ventral tegmental area (VTA) induces a marked behavioural hyperactivity (13, 14, 17). This response is presumably mediated via an activation of the A10 dopaminergic cell bodies located within the VTA since selective lesion with 6-hydroxydopamine prevents the response (14,17). In addition to the natural agonist, the relatively stable substance P analogue, [pGlu^5, MePhe^8, Sar^9] substance P_{5-11} (DiMe-C7) similarly evokes an increase in locomotor activity (7, 8, 11, 12), which is associated with increases in catecholamine-related neurochemical levels in selective forebrain regions such as the nucleus accumbens (8, 11, 12). This may have major relevance to the increase in behavioural activity since similar observations are noted following the discrete injection/infusion of exogenous dopamine into this limbic nucleus (4,5). However, in addition to the nucleus accumbens, other nuclei may also be implicated in the increase in locomotor activity (2) caused by the local injection of DiMe-C7 into the VTA. In the present studies additional evidence is obtained that DiMe-C7 can induce neurochemical changes in various limbic and extrapyramidal nuclei which may contribute to changes in behavioural activity.

METHOD

Female Hooded Lister rats (225–275 g, Bradford strain) were anaesthetised (pentobarbitone/chloral hydrate mixture; 30/150 mg·kg^{-1} IP respectively) and implanted with chronically indwelling stainless steel guide cannulae (0.65 mm external diameter) terminating 7 mm above the VTA. Following a 14-day recovery period, cannulated animals received a bilateral injection of DiMe-C7 (3.0 μg into each VTA in a volume of 0.5 μl injected over a 1-min period) or vehicle (N_2 bubbled 0.9% wt./vol. sodium chloride) via stainless steel injection units (0.3 mm external dia.) inserted down the guide cannulae and terminating in the VTA [Ant. 2.8, Vert. −3.5, Lat. ±1.0, (15)]. Following injection of DiMe-C7 into the VTA, animals were either monitored for 90 min in perspex locomotor activity boxes equipped with photocell units (25 × 15 × 15 cm high) or killed at 15 min when the brains were removed and various nuclei dissected for the quantitation of indoleamine- and catecholamine-related neurochemicals by HPLC with electrochemical detection by the method of Barnes and colleagues (1).

Following the behavioural experiments or the dissection for neurochemical determinations, the intact mesencephalon was pre-

TABLE 1

ALTERATIONS IN INDOLEAMINE- AND CATECHOLAMINE-RELATED NEUROCHEMICALS IN VARIOUS RAT FOREBRAIN NUCLEI 15 MIN FOLLOWING THE BILATERAL INTRA-VTA INJECTION OF DiMe-C7 (6.0 μg/1.0 μl) OR VEHICLE (0.9% wt./vol. SODIUM CHLORIDE)

	DA	DOPAC	HVA	NA	5-HT	5-HIAA
Striatum, Veh	11316 ± 890	1323 ± 70	879 ± 75	62 ± 7	142 ± 13	120 ± 21
Striatum, DiMe-C7	7916 ± 359† (−30%)	1636 ± 91* (+24%)	818 ± 108 (−7%)	56 ± 4 (−10%)	134 ± 9 (−6%)	106 ± 4 (−12%)
Nucleus accumbens, Veh	5108 ± 372	1476 ± 121	655 ± 56	215 ± 21	154 ± 15	191 ± 22
Nucleus accumbens, DiMe-C7	5382 ± 387 (+5%)	2473 ± 276† (+68%)	875 ± 56* (+34%)	266 ± 32 (+24%)	112 ± 13 (−27%)	210 ± 24 (+10%)
Tuberculum olfactorium, Veh	1477 ± 85	174 ± 16	285 ± 30	76 ± 4	227 ± 29	106 ± 12
Tuberculum olfactorium DiMe-C7	1556 ± 83 (+5%)	186 ± 14 (+7%)	380 ± 60 (+33%)	98 ± 7* (+29%)	301 ± 17 (+33%)	130 ± 18 (+23%)
Amygdala,Veh	498 ± 62	288 ± 29	77 ± 9	583 ± 49	614 ± 49	519 ± 46
Amygdala, DiMe-C7	790 ± 93* (+59%)	324 ± 19 (+13%)	99 ± 9 (+29%)	581 ± 39 (0%)	666 ± 46 (+8%)	597 ± 30 (+15%)
Entorhinal cortex, Veh	19 ± 2	19 ± 3	24 ± 3	233 ± 17	238 ± 34	90 ± 11
Entorhinal cortex, DiMe-C7	27 ± 3 (+42%)	28 ± 2* (+47%)	55 ± 9† (+129%)	428 ± 36‡ (+84%)	259 ± 54 (+9%)	151 ± 38 (+68%)
Frontal cortex, Veh	81 ± 5	34 ± 5	30 ± 5	317 ± 14	301 ± 26	220 ± 17
Frontal cortex, DiMe-C7	85 ± 8 (+5%)	48 ± 6 (+41%)	39 ± 7 (+30%)	416 ± 33* (+31%)	375 ± 35 (+25%)	140 ± 12† (−36%)
Hippocampus, Veh	ND	ND	ND	208 ± 29	130 ± 9	132 ± 9
Hippocampus, DiMe-C7	ND	ND	ND	351 ± 47 (+69%)	115 ± 9 (−12%)	134 ± 7 (+2%)
Septum,Veh	113 ± 16	75 ± 11	ND	158 ± 14	103 ± 10	92 ± 9
Septum, DiMe-C7	116 ± 13 (+3%)	85 ± 12 (+13%)	ND	160 ± 14 (+1%)	96 ± 8 (−7%)	97 ± 8 (+5%)

Data represent mean ± S.E.M. (pg/mg wet weight), n = 9–12. % changes are indicated in parentheses.
Significant alteration in neurochemical level following DiMe-C7 injection, *$p<0.05$, †$p<0.01$ and ‡$p<0.001$ (Student's t-test).
ND: neurochemical level below the limit of detection.

served for the histological verification of the injection placement. Data from animals receiving injections outside of the VTA were not included in the present report.

RESULTS

The bilateral administration of DiMe-C7 (6.0 μg) into the VTA initiated a marked increase in locomotor activity which peaked at around 15 to 30 min and lasted for up to 60 min (Fig. 1).

Ex vivo neurochemical analysis 15 min following the injection of DiMe-C7 (6.0 μg) identified a significant increase in the levels of dopamine and/or its metabolites (3,4-dihydroxyphenylacetic acid and homovanillic acid) in the nucleus accumbens, entorhinal cortex, amygdala and striatum ($p<0.05$–0.001, Student's t-test, Table 1). In addition, noradrenaline levels were increased in the tuberculum olfactorium, frontal cortex and entorhinal cortex ($p<0.05$–0.001, Student's t-test, Table 1), whilst 5-hydroxyindoleacetic acid levels were increased in the frontal cortex ($p<0.01$, Student's t-test, Table 1).

DISCUSSION

Consistent with previous reports (7, 8, 11, 12), the present studies have shown that a behavioural hyperactivity occurs in rats following the intra-VTA administration of DiMe-C7. Furthermore, in addition to the previously documented increase in catecholamine metabolism in the nucleus accumbens, neurochemical levels were altered in other limbic and extrapyramidal brain areas. These findings have physiological relevance since the VTA receives a substance P innervation originating from the habenula (6,9).

The VTA contains the A10 catecholamine (dopamine) cell bodies which project to predominantly limbic forebrain nuclei (nucleus accumbens, tuberculum olfactorium, amygdala, frontal cortex, entorhinal cortex, septum), stimulation of which presumably results in the neurochemical changes within some of these terminal regions. However, a significant innervation to the striatum also exists (10,16), which may account for the increase in dopamine metabolism in the striatum. However, the possibility must also be considered that, due to the close proximity of the VTA and substantia nigra compacta, the DiMe-C7 may have diffused to the substantia nigra compacta to directly stimulate the nigral-striatal dopaminergic pathway (7).

Although it is consistent with previous studies to hypothesise that an increase in dopaminergic activity within the nucleus ac-

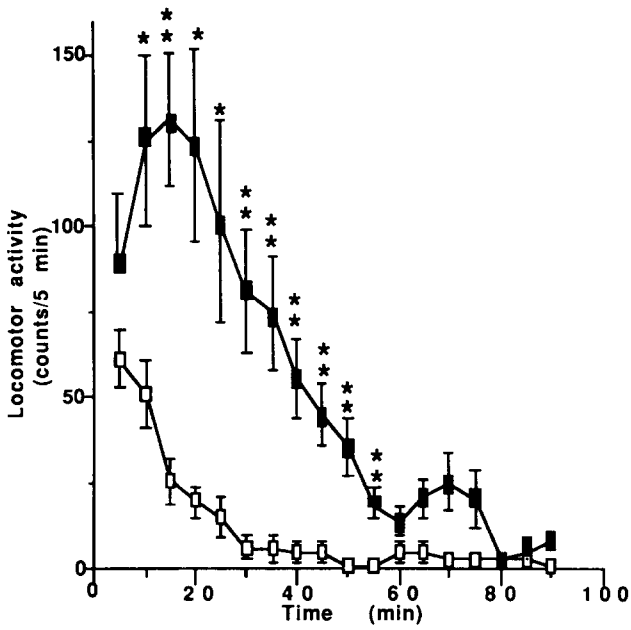

FIG. 1. Behavioural consequence of bilateral intra-VTA injection of DiMe-C7 (6.0 μg, ■) or vehicle (0.9% wt./vol. sodium chloride, □). Data represent mean ± S.E.M., n = 5–6. Significant difference between DiMe-C7- and vehicle-induced response, *$p<0.05$, **$p<0.01$ (Student's t-test).

cumbens results in hyperactivity (4,5), the detection of an increase in dopamine levels in the amygdala may also be relevant, since the injection of dopamine into this area similarly evokes an increase in locomotor activity (2). Although previously unreported, the alteration in amygdaloid dopamine levels following intra-VTA DiMe-C7 was not entirely unexpected since increases in dopamine activity have been reported in this nucleus following excitation of the VTA with discrete injections of neurotensin (3). Also, more recently, DiMe-C7-induced VTA stimulation has been reported to unilaterally increase amygdaloid dopamine metabolism (12).

In summary, the present studies have shown that behavioural hyperactivity following intra-VTA administration of DiMe-C7 is accompanied by marked increases, mainly in catecholamine-related neurochemical levels, in various limbic and extrapyramidal forebrain nuclei. It is proposed that in addition to the nucleus accumbens, other nuclei may be involved in the mediation of the hyperactivity response.

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