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Behavioural and biochemical effects of substance P injected into the substantia nigra of the rat

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Increasing biochemical and immunocytochemical evidence suggests that the undecapeptide substance P (SP) may function as a neurotransmitter in the mammalian cns (Konishi & Otsuka, 1975; Cuello, Polak & Pearse, 1976). In the rat there is strong evidence for pathways utilizing SP as a transmitter descending from the caudate nucleus and globus pallidus to the substantia nigra, where SP is found in the highest concentrations in the zona reticulata (Kanazawa & Jessell, 1976; Kanazawa, Emson & Cuello, 1977). Depolarizing stimuli have been shown to release SP from slices of SN tissue in a calcium-dependent fashion (Jessell, 1977), whilst Davies & Dray (1976) found that microiontophoretically-applied SP excited nigral neurons. It is possible, therefore, that the peptide from this striatonigral pathway normally acts to control the firing rate of the reciprocal ascending nigrostriatal dopaminergic neurons. To test this suggestion pure synthetic SP was injected unilaterally into the rat's substantia nigra and the animals observed subsequently for evidence of rotational locomotor activity characteristic of nigrostriatal activation (Ungerstedt, 1971). The concentrations of dopamine and its major metabolite homovanillic acid (HVA) present in the corresponding corpora striata were determined as a biochemical measure of dopaminergic cell activity.

Male Wistar albino rats (Tuck), 200–250 g, were lightly anaesthetized with a mixture of oxygen, nitrous oxide and halothane. One ng of SP was injected stereotactically in a volume of 0·1 μl into the right substantia nigra using a 1 μl Hamilton syringe. Control animals received an equivalent volume of physiological saline. On recovery, animals were placed in a rectangular box and observed for rotational and other stereotyped behaviour. Ten min after injection the rats were stunned and decapitated and the brains quickly removed and frozen in a solid CO_2/methanol mixture. The corpora striata from both hemispheres were subsequently dissected out according to the method of Glowinski & Iversen (1966) and assayed fluorimetrically for dopamine (Shellenberger & Gordon, 1971) and HVA (Murphy, Robinson & Sharman, 1969). The sites of all injections were determined histologically.

As little as 1 ng SP was sufficient to induce circling movement in rats. Table 1 shows that the direction of this turning response was dependent on the site of injection and correlated with nigrostriatal dopamine cell activity. Injections into the zona reticulata invariably elicited contralateral turning which reached a peak frequency of approximately 4 rotations min^{-1} at 7 min after injection, but which faded by 10 min. The short duration may have been caused by breakdown of the SP by peptidases. Locomotion was typically slow and intermittent and was frequently

Table 1. *Direction of rotation and changes in striatal HVA concentrations induced by SP in the rat.*

Dose of SP (ng)	n	Injection site	Rotation	Mean striatal HVA levels (μg g^{-1} wet wt) Left	Right	Diff. (%)
1	9	Zona reticulata	Contra-lateral	0.409	0.660	+36.9*
1	7	Anterior or dorsal to zona recticulata	Ipsilateral	0.695	0.551	−20.7*
0	7	All areas Injection	None	0.503	0.479	− 4.7

All injections were given in a volume of 0.1 μl.
S.e.m. all in the range ±6–10%.
* $P < 0.001$ by paired t-test.

* Correspondence.

interrupted by periods of exaggerated gnawing and sniffing. On the other hand, when the SP was injected either immediately dorsal to, or anterior to the zona reticulata the animals always responded with a postural bias or turning in the opposite direction. SP injections made posterior to the zona reticulata, or injections of saline given into any of these regions, failed to elicit any form of stereotyped behaviour.

Although there was considerable variation between animals in the striatal concentrations of dopamine (range 0·9–1·9 $\mu g\,g^{-1}$ wet wt) and HVA (range 0·32–1·02 $\mu g\,g^{-1}$ wet wt), bilateral differences in the striatal contents of these substances in saline-injected controls were less than 10%. In the low dose employed here SP had no effect on the steady-state level of dopamine in the striatum. However, Table 1 clearly indicates that SP-induced contralateral turning was accompanied by a pronounced increase in ipsilateral striatal HVA concentrations ($P < 0.001$), and that turning towards the injected side was characterized by a significant decrease in this metabolite ipsilaterally ($P < 0.001$). SP injections at sites which did not elicit turning caused no significant change in striatal HVA concentrations.

Since the neurons in the substantia nigra are known to be susceptible to excitation by electrophoretically-applied SP (Davies & Dray, 1976), it seems reasonable to speculate that the SP-induced contralateral turning observed here results from the unilateral stimulation of the ascending nigrostriatal dopaminergic pathways, especially as the HVA concentrations in the corresponding striata were raised accordingly.

The critical placement of the SP injection is also interesting, because it emphasises that the application of SP to other cells in the neighbourhood of the zona reticulata ultimately reduces impulse traffic in the ascending dopaminergic neurons on that side and leads to ipsilateral circling. The nature of the synaptic connections in the region of the substantia nigra is not fully understood, but it is possible that in this case SP may be acting indirectly upon the zona reticulata through the intervention of an inhibitory interneuron.

Although the mode of action of SP remains to be determined, this preliminary study serves to illustrate that SP possesses demonstrable physiological activity when injected in small doses into the substantia nigra, an area of the brain which possesses high concentrations of SP and which could conceivably utilize this peptide as an endogenous synaptic transmitter.

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A simplified decerebration technique in cats and its applicability to neuro-cardiovascular drug studies†

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Elimination of higher central nervous system (cns) structures by decerebration has been carried out by a variety of techniques. However, these methods are not entirely satisfactory as they either require specialized instrumentation (Kent, Drane & Manning, 1971) result in massive trauma and blood loss (Chai & Wang, 1962) or do not completely eliminate higher cns influence on hind brain activity (Borison, Clark & Rosenstein, 1963). The purpose of our work was to develop a technique that would avoid these problems.

We modified a stainless steel microspatula (spatula, micro, stainless steel, mirrowed finish, No. 57949-022, VWR Scientific) into an instrument that could be

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