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Ventral tegmental area: site through which dopamine D_2-receptor agonists evoke behavioural and electrocortical sleep in rats

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1 In freely moving rats the effects on behaviour and electrocortical (ECoG) spectrum power of some dopamine agonists, i.e. apomorphine and (+)-3PPP, given directly into different areas of the rat brain were studied. In particular, dopamine agonists were microinfused in the ventral tegmental area (VTA) and substantia nigra (SN) or into the caudate nucleus, n. accumbens and prefrontal cortex. The ECoG spectrum power effects were continuously analysed by means of a computerized Berg-Fourier analyser as total spectrum power and power in preselected frequency bands.

2 Apomorphine and (+)-3PPP (0.01, 0.1 and 1.0 nmol) given bilaterally into the VTA produced behavioural and ECoG sleep in a dose-dependent fashion. A statistically significant ($P < 0.01$) increase in ECoG total spectrum power with a predominant increase in the lower frequency bands (0.25–3, 3–6 and 6–9 Hz) occurred. No behavioural and ECoG changes were evoked by the same doses of apomorphine bilaterally microinfused into the SN or into the caudate nucleus or by (+)-3PPP (1.0 nml) microinjected into the n. accumbens or applied onto the prefrontal cortex.

3 Behavioural and ECoG sleep was also induced in rats after systemic administration of apomorphine (263 nmol kg^{-1}, i.p.).

4 The behavioural and ECoG spectrum power effects of apomorphine (1.0 nmol) bilaterally microinfused into the VTA were prevented by a previous microinjection into the same site of (−)-sulpiride (9.8 nmol). Similarly, behavioural and ECoG effects evoked by (+)-3PPP (0.1 nmol) given bilaterally into the VTA, were completely antagonized by a previous injection into the same site of haloperidol (16 pmol given 10 min before). In contrast, pretreatment with SCH 23390 (50 $\mu g\,kg^{-1}$, s.c.), a selective antagonist at dopamine D_1-receptors, was unable to antagonize the behavioural and ECoG spectrum power effects of (+)-3PPP.

5 Soporific effects induced by systemic administration of apomorphine were antagonized by (−)-sulpiride (9.8 nmol) given bilaterally into the VTA 10 min before, whereas, yohimbine (1.3 nmol), (an antagonist at α_2-adrenoceptors) bilaterally microinfused into the VTA, was ineffective in this respect.

6 The present experiments provide evidence suggesting that stimulation of dopamine D_2-receptors located at the cell body level and/or the dendrites of dopaminergic neurones in the VTA may represent the mechanism through which apomorphine or (+)-3PPP exert their soporific effects in rats.

Introduction

A large body of experimental evidence suggests a role for dopamine in the control of sleep-arousal mechanisms (see Koella, 1984; Wauquier *et al.*, 1985). Apomorphine, a dopamine receptor agonist, given systemically produces in mammals biphasic effects on behaviour and ECoG activity, low (non-emetic) doses producing sleep, larger doses producing behavioural arousal and ECoG desynchronization (Di Chiara *et al.*, 1976; Kafi & Gaillard, 1976; Mereu *et al.*, 1979). The soporific effects of apomor-

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phine are mediated by central structures as suggested by experiments in which this compound was given into specific areas of the brain (Bagetta *et al.*, 1987a). Moreover, domperidone, a selective dopamine receptor antagonist which does not cross the blood brain barrier (Laduron & Leysen, 1979), failed to antagonize the typical low dose effects of apomorphine (Gessa *et al.*, 1985). Soporific effects of low doses of apomorphine seem to be linked to the preferential stimulation of dopamine presynaptic receptors resulting in inhibition of dopamine synthesis and release (Carlsson, 1975; Di Chiara *et al.*, 1976; Argiolas *et al.*, 1982), whereas behavioural arousal and ECoG desynchronization seem to be due to activation of postsynaptic dopamine receptors in the brain (Andén *et al.*, 1970; Strömbon, 1976).

With the introduction of new drugs acting as selective agonists or antagonists at dopamine postsynaptic D_1- and D_2-receptors or at dopamine presynaptic autoreceptors there has been a renewal of interest in the role played by the two dopamine receptor subtypes in the control of sleep-arousal mechanisms. In fact, we have recently reported that the i.c.v. injection of R-(−)-3(3-hydroxy-phenyl-n-propyl-piperidine hydrochloride ((−)-3PPP), a specific agonist at dopamine autoreceptors (Hjorth *et al.*, 1981) produced in freely moving rats dose-dependent behavioural and ECoG sleep (Bagetta *et al.*, 1987b). In favour of a presynaptic site for the mediation of soporific effects of (−)-3PPP and of small doses of apomorphine is the antagonism of these effects by selective antagonists at D_2-receptors, i.e. (−)-sulpiride and small doses of haloperidol (Gessa *et al.*, 1985; Bagetta *et al.*, 1987b). The present study was designed to ascertain the site through which the behavioural and ECoG sleep induced by apomorphine and (+)-3PPP were mediated. Thus the behavioural and ECoG effects of apomorphine and (+)-3PPP, which in comparison with the (−)-isomer is reputed to be a full agonist at D_2-dopamine receptors (Hjorth, 1983), were studied in rats after microinjection into several brain areas rich in dopamine cell bodies, i.e. ventral tegmental area (VTA), substantia nigra (SN) or into areas containing dopaminergic nerve endings, i.e. caudate nucleus, prefrontal cortex, n. accumbens. In addition, we planned to ascertain whether behavioural and ECoG sleep induced by systemic administration of apomorphine was antagonized by selective dopamine antagonists given directly into some specific areas of the brain.

Methods

Adult male Wistar rats (200 ± 20.2 g) housed in stable conditions of humidity (65%) and temperature (22 ± 2°C), with a reversed dark-light cycle (08 h 00 min–20 h 00 min light off), were used. Under chloral hydrate (400 mg kg^{-1}, i.p.) anaesthesia, animals were placed in a stereotaxic apparatus (David Kopf) and bilaterally implanted with stainless steel guide cannulae (25 gauge) into the VTA, SN (pars compacta), caudate n., n. accumbens and prefrontal cortex, according to the atlas of Paxinos & Watson (1982). One week was allowed after surgery before experiments were carried out. Electrocortical (ECoG) activity was recorded by use of an 8 channel ECoG machine (OTE Biomedica, Florence) via 4 chronically implanted steel screw electrodes onto each fronto-parietal cortex, with a stereotaxic instrument guide (2 and 4 mm behind the bregma and 2 mm laterally to the midline). Quantification of ECoG total voltage power (0.25–16 Hz) and of preselected frequency bands was obtained by means of a computerized Berg-Fourier analyzer (OTE Biomedica) according to Bricolo *et al.* (1978). ECoG power spectra were derived from 5 min ECoG epochs and the integrated energy signals were expressed as $\mu V^2 s^{-1}$; the time constant (0.03 s) was short enough to reduce the number of the artefacts (HF cut-off = 5.3 Hz). For dose-response curves, regression lines were calculated by least square analysis and correlation coefficients (r) between these were calculated.

The intracerebral microinjections were carried out with a 1 μl Hamilton microsyringe connected by a teflon tube to an injector cannula (volume of infusate 0.5 μl per side). Control infusions were made with the same volume of vehicle and did not affect behaviour and ECoG spectrum power.

Before testing, animals were individually placed in a perspex box and allowed 10 min to acclimatize to the new environment. Each rat was treated only once. Histological examination post-mortem confirmed the location of the guide cannula.

All data are represented as means ± s.e.mean. Statistically significant differences between control and post-treatment data, obtained for the same interval periods, were calculated with Student's t test. ED_{50} values and confidence limits for percentage increase in effects after drug treatments on total voltage spectrum power were evaluated by probit analysis by use of a computer programme described by Tallarida & Murray (1981). The number of rats is given in parentheses.

Drugs

Apomorphine HCl (mol. wt. 303.8; Sigma, USA) was dissolved in twice distilled water and few drops of a 0.5% solution of ascorbic acid were added; (−)-sulpiride (Ravizza S.p.A., Muggiò, Milan) and haloperidol (Lusofarmaco, Milan) were used.

(+)-3PPP [R(+)-3 (3 hydroxy-phenyl-n-propyl-piperidine)] hydrochloride (mol. wt. 255.79); SCH 23390 [R-(+)-8 (chloro-2,3,4,5 tetrahydro-3-methyl-5-phenyl-1 H-3-benzazepine-7-ol)] (mol. wt. 324.11; R.B. Inc., MA, USA) and yohimbine HCl (mol. wt. 354.43; SIGMA, USA) were dissolved in twice distilled water.

Results

Behavioural and ECoG spectrum power effects of apomorphine given into the ventral tegmental area

The bilateral microinjection of apomorphine (0.01, 0.1 and 1.0 nmol) into the rat VTA produced behavioural and ECoG sleep in a dose-dependent ($r = 0.89$) fashion. In particular, a dose of 1.0 nmol ($n = 8$) produced behavioural sedation and sleep lasting 72.5 ± 9.46 min, accompanied by a statistically significant ($P < 0.01$) increase in total voltage power and in the lower frequency (0.25–3, 3–6 and 6–9 Hz) bands. A typical example is shown in Figure 1.

Similar but shorter-lasting (50 ± 15.81 min) behavioural and ECoG spectrum power effects were observed after a lower dose (0.1 nmol; $n = 8$). A dose of 0.01 nmol ($n = 6$) did not affect behaviour and ECoG spectrum power activity. (−)-Sulpiride, bilaterally microinjected into the VTA ($n = 6$) in a dose (9.8 nmol) which *per se* did not affect behaviour and ECoG spectrum power was able to prevent behavioural and ECoG spectrum power effects induced by apomorphine (1.0 nmol) given 10 min later into the same site.

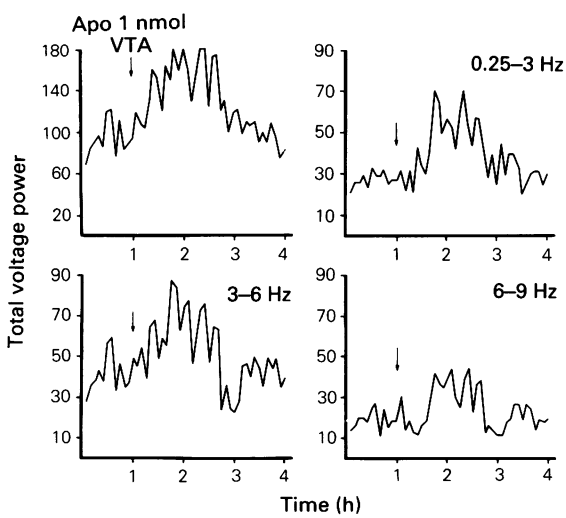

Figure 1 Typical example of ECoG spectrum power effects induced by apomorphine (Apo) (1.0 nmol) given bilaterally into the ventral tegmental area (VTA). Note the sustained increase in ECoG total voltage power ($\mu V^2 s^{-1}$) as well as in lower frequency bands, (0.25–3, 3–6 and 6–9 Hz).

Bilateral microinjection of apomorphine (0.1 and 1.0 nmol; $n = 8$ for each dose) into the SN or into the caudate n. produced only a slight behavioural sedation without concomitant changes in ECoG spectrum power. In addition, the microinjection of apomorphine in the locus coeruleus, an area where noradrenaline cell bodies are located, did not produce significant behavioural and ECoG changes.

Behavioural and ECoG spectrum power effects after systemic administration of apomorphine

In freely moving rats, the systemic administration of a low dose (263 nmol kg^{-1}, i.p.) of apomorphine produced, after a brief latency period (11.3 ± 0.6 min), in which a slight increase in locomotor activity and tachypnoea occurred, a longer-lasting phase (34.10 ± 1.8 min) of behavioural sedation or sleep occasionally interrupted by yawning.

These effects were accompanied by ECoG synchronizing phenomena with a statistically significant ($P < 0.01$) increase in total voltage power and in lower frequency (0.25–3, 3–6 and 6–9 Hz) bands.

(−)-Sulpiride, bilaterally microinjected into the VTA ($n = 8$) but not in other areas (caudate n., prefrontal cortex, s. nigra) ($n = 4$ experiments for each area) in a dose (9.8 nmol, 10 min before) which *per se* did not affect behaviour and ECoG spectrum power, was able to antagonize the behavioural and ECoG sleep typically induced by subsequent intraperitoneal administration of apomorphine (263 nmol kg^{-1}) (Figure 2).

In contrast, pretreatment with an antagonist at α_2-adrenoceptors, yohimbine (1.3 nmol; $n = 6$) bilaterally microinjected into the VTA, did not affect the soporific effects produced by apomorphine (263 nmol kg^{-1} i.p. given 10 min later), showing that apomorphine effects are specifically mediated through D_2-dopamine receptors. This dose of yohimbine was able to prevent behavioural and ECoG sleep induced by clonidine, given into the locus coeruleus (De Sarro *et al.*, 1987).

Injection into the ventral tegmental area of (+)-3PPP

The bilateral injection into the VTA of (+)-3PPP (0.01, 0.1 and 1.0 nmol), produced dose-dependent behavioural sedation or sleep and an increase in the ECoG spectrum power with a predominant increase in 0.25–3, 3–6 and 6–9 Hz frequency bands. The dose of 0.01 nmol ($n = 6$) was unable to affect in a significant manner either behaviour or ECoG spectrum

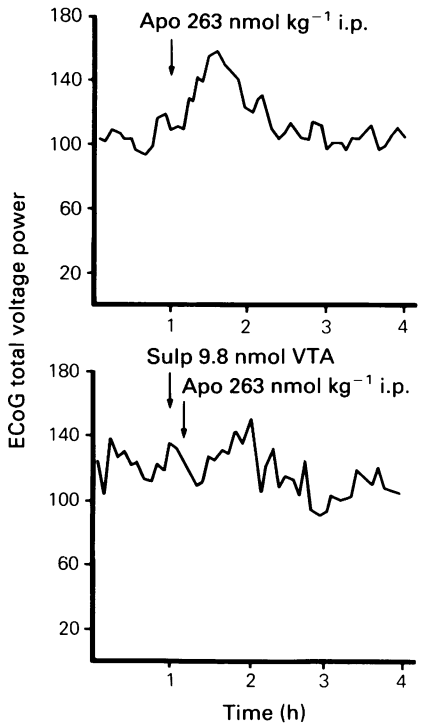

Figure 2 Antagonism by (−)-sulpiride (Sulp) (9.8 nmol), bilaterally microinjected into the ventral tegmental area (VTA), of the ECoG spectrum power ($\mu V^2 s^{-1}$) effects induced by intraperitoneal administration of apomorphine (Apo) (263 nmol kg^{-1}), given 10 min later.

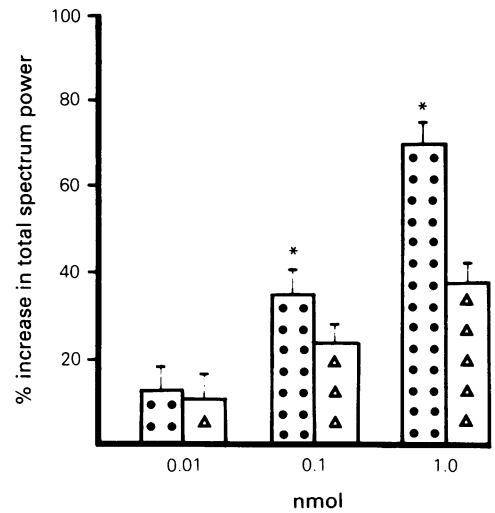

Figure 3 Dose-dependent increase in ECoG total spectrum power ($\mu V^2 s^{-1}$) in rats 60 min after bilateral microinjection into the ventral tegmental area (VTA) of (+)-3PPP (●) ($r = 0.95$) and apomorphine (△) ($r = 0.89$). The number of experiments was as follows: $n = 6$ (0.01 nmol), $n = 8$ (0.1 and 1.0 nmol). The ED_{50} of (+)-3PPP in inducing increase in ECoG total spectrum power was 0.25 nmol (0.068–0.91). (+)-3PPP was 10 times more powerful than apomorphine in producing the same percentage increase of ECoG total spectrum power. * $P < 0.01$ vs. apomorphine.

power. However, higher doses (0.1 and 1.0 nmol; $n = 8$ for each dose) produced dose-dependent behavioural sedation and slow-wave sleep with spindles lasting 61.25 ± 1.25 min or 111.25 ± 6.25 min, according to the dose. In addition, a dose-dependent ($r = 0.95$) increase in the ECoG total voltage power was observed, the most significant ($P < 0.01$) increase occurring in the lower frequency bands (0.25–3, 3–6 and 6–9 Hz). The ECoG spectrum power increase elicited after (+)-3PPP microinfused into the VTA was significantly higher in comparison to corresponding doses of apomorphine (Table 1 and Figure 3). No behavioural and ECoG spectrum power effects were produced by (+)-3PPP (1.0 nmol) microinjected into the n. accumbens or applied onto the prefrontal cortex ($n = 4$). In Table 2 the effects on ECoG spectrum power of (+)-3PPP in comparison to apomorphine given into different areas of the brain are shown.

Table 1 Comparative effects of R(+)-3(3 hydroxy-phenyl-n-propyl-piperidine)hydrochloride ((+)-3PPP) and apomorphine, bilaterally microinfused into the ventral tegmental area, on ECoG total voltage power ($\mu V^2 s^{-1}$) in rats

Drug	*Dose* (nmol)	n	*Control period*	*30 min*	*60 min*	*90 min*
(+)-3PPP	0.01	6	82.6 ± 4.6	89.4 ± 7.9	93.3 ± 3.2	84.2 ± 5.2
	0.1	8	90.9 ± 2.0	103.4 ± 2.7**	124.2 ± 9.9*	97.0 ± 6.6
	1.0	8	91.6 ± 5.5	128.1 ± 14.1**	159.5 ± 14.5*	109.1 ± 11.1
Apomorphine	0.01	6	61.6 ± 2.0	66.5 ± 3.8	68.5 ± 7.5	59.8 ± 1.4
	0.1	8	79.1 ± 2.1	88.0 ± 2.6**	97.5 ± 2.4*	84.6 ± 4.0
	1.0	8	56.3 ± 3.4	68.6 ± 4.1**	76.7 ± 5.3*	58.6 ± 6.8

* $P < 0.01$; ** $P < 0.05$ vs. control values. n = number of experiments.

Table 2 Effects of R(+)-3(3-hydroxy-phenyl-n-propyl-piperidine) hydrochloride ((+)-3PPP) and apomorphine on the ECoG total voltage power ($\mu V^2 s^{-1}$) after microinfusion into some areas of the rat brain

Drug and brain areas	n	*Control period*	*30 min*	*60 min*	*90 min*
(+)-3PPP (1.0 nmol)					
VTA	8	91.6 ± 5.5	128.1 ± 14.1**	159.5 ± 14.5*	109.1 ± 11.1
Accumbens	4	83.2 ± 3.8	80.5 ± 4.4	85.0 ± 3.6	77.3 ± 2.2
Prefrontal cortex	4	88.0 ± 4.0	94.7 ± 2.9	93.0 ± 3.3	86.7 ± 3.3
Apomorphine (1.0 nmol)					
VTA	8	56.3 ± 3.4	68.6 ± 4.1**	76.7 ± 5.3*	58.6 ± 6.8
S. Nigra	8	95.3 ± 2.7	98.5 ± 1.7	96.3 ± 3.6	92.1 ± 2.9
Caudate	8	78.0 ± 3.0	72.6 ± 2.4	84.0 ± 4.2	81.6 ± 4.3

* $P < 0.01$; ** $P < 0.05$ vs. control values. n = number of experiments.

The behavioural and ECoG synchronizing effects produced by (+)-3PPP (0.1 nmol; $n = 8$) were completely antagonized by haloperidol (16 pmol), given into the same site (Figure 4) in a dose, which *per se* did not affect behaviour and ECoG spectrum power. On the contrary, systemic pretreatment (20 min before) with SCH23390, a selective antagonist at dopamine D_1-receptors (Hyttel, 1983; Iorio *et al.*, 1983); at a dose (50 $\mu g\,kg^{-1}$, s.e.) level which *per se* did not affect behaviour and ECoG sepctrum power, was unable to antagonize the behavioural and ECoG spectrum power effects elicited by the intermediate dose of (+)-3PPP (n = 6) given into the VTA.

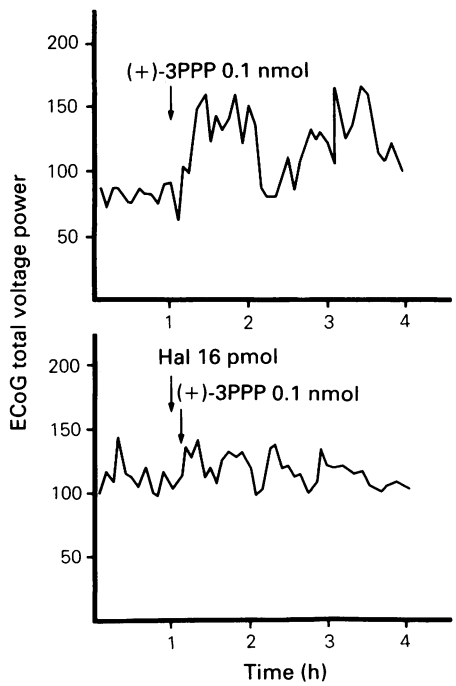

Figure 4 Antagonism by pretreatment (10 min before) with haloperidol (Hal) (16 pmol) given bilaterally into the ventral tegmental area (VTA) of the ECoG spectrum power ($\mu V^2 s^{-1}$) effects elicited by (+)-3PPP (0.1 nmol) microinjected into the same site.

Discussion

The present experiments confirm that dopaminergic systems in the brain participate in the control of sleep arousal mechanisms (see Koella, 1984; Wauquier *et al.*, 1985). In addition, they provide evidence that the VTA represents the main site through which dopamine receptor agonists produce their soporific effects. In fact, when apomorphine was given in another area containing dopamine cell bodies (i.e. SN) only slight behavioural sedation, without significant changes in ECoG spectrum power, occurred. Similarly, no behavioural and ECoG spectrum power changes were observed in rats receiving apomorphine or (+)-3PPP in brain areas rich in dopaminergic terminals, i.e. n. accumbens, caudate nucleus and prefrontal cortex. It is well known that VTA contains dopamine cell bodies from which axons project to cortical and limbic regions (dopaminergic mesocortical and mesolimbic systems) (see Dahlstrom & Fuxe, 1964; Björklund & Lindvall, 1984; Oades & Halliday, 1987). Electrophysiological evidence indicates that dopamine and dopamine D_2-receptor agonists (i.e. N-propyl norapomorphine, apomorphine, lisuride, pergolide, LY141865, bromocriptine) inhibit the firing rate of VTA dopamine cell bodies (Trulson & Precussler, 1984; Wang *et al.*, 1987). Thus, it is conceivable that

apomorphine and (+)-3PPP directly applied into the VTA, act by stimulating dopamine D_2-autoreceptors located on the membrane cell bodies and/or on the dendrites of dopaminergic neurones thereby inhibiting dopamine cell bodies firing rate and decreasing the activity of mesolimbic and mesocortical pathways. The present results indicate that a focal inhibition of dopaminergic transmission following stimulation of D_2-receptors located at the nerve endings is not sufficient to produce behavioural and electrocortical sleep, this effect being obtained when the whole mesocortical and/or mesolimbic dopaminergic systems are inhibited. In addition, the finding that (−)-sulpiride, given directly into the VTA, antagonizes the behavioural and ECoG sleep induced by systemic apomorphine strengthens the idea that this dopaminergic area and not others is responsible for mediating sleep induced by dopaminomimetic compounds.

The specificity of dopamine D_2-receptors in mediating apomorphine-induced behavioural and ECoG sleep is confirmed by experiments showing that yohimbine, a selective α_2-adrenoceptor antagonist, and SCH 23390, an antagonist at dopamine D_1-receptors, were unable to antagonize the soporific effects of apomorphine. From previous experiments it was clear that behavioural and ECoG sleep were obtained after i.c.v. injection of selective antagonists of D_1-(SCH23390) and D_2-(haloperidol) postsynaptic receptors, thus enhancing the concept that pharmacological manipulations leading to a decrease of dopaminergic transmission, either acting at the presynaptic level (dopamine autoreceptor stimulation) or at the postsynaptic level (D_1- and D_2-receptor antagonists) give rise to sedation and sleep (see Longo, 1978, Bagetta *et al.*, 1987b,c; Wauquier *et al.*, 1985). On the contrary, drugs acting by potentiating dopaminergic transmission either acting as dopamine D_1- and D_2-postsynaptic agonists, as precursors (L-DOPA) or by releasing catecholamines or inhibiting catecholamine reuptake, i.e. (+)-amphetamine, produce behavioural arousal and ECoG desynchronization (see Marley & Nisticò, 1972; Monti, 1982; Ongini *et al.*, 1985; Wauquier *et al.*, 1985; Wang *et al.*, 1987). In addition, the activation of adenylate cyclase by cholera toxin produces behavioural stimulation and ECoG desynchronization (Nisticò *et al.*, 1976) thus reinforcing the concept that stimulation of dopamine D_1-receptors (linked to adenylate cyclase) participates in the behavioural and ECoG arousal.

Dopaminergic neurones in the VTA do not seem to be tonically inhibited by dopamine D_2-autoreceptors, since (−)-sulpiride itself given bilaterally into this area did not affect behaviour and ECoG spectrum power; this finding is different from that reported in the rat at the locus coeruleus level where during the day, α_2-autoreceptors seem to be tonically active, since behavioural arousal and ECoG desynchronization occur after microinjection into this area of yohimbine, an α_2-adrenoceptor antagonist (De Sarro *et al.*, 1987).

In conclusion, the present experiments show that with pharmacological manipulations in specific areas of the rat brain, by means of selective agonists and antagonists at dopamine D_2-receptors, the site through which dopamine (data not reported) and dopamine receptor agonists produce behavioural sleep and ECoG synchronization is represented by the VTA. In addition, they provide evidence that the stimulation of dopamine D_2-receptors located at the cell body level and/or on the dendrites of dopaminergic neurones in this area seems to be the mechanism through which apomorphine and other dopamine receptor agonists exert their soporific effects.

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