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Role of the dopaminergic system in the cataleptogenic action of bulbocapnine

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Time courses of the behavioural and biochemical effects of a cataleptogenic dose (50 mg kg^{-1} i.p.) of bulbocapnine have been studied in the rat. Catalepsy ensues immediately after administration of the drug and lasts 1 h. Concomitant with the onset of catalepsy there is a rise in HVA and DOPAC concentrations in whole and discrete parts of the brain (striatum, limbic system). Dopamine content does not change in whole brain but it decreases in the striatum and increases in the cortex and hippocampus. No significant effects on NA, 5-HT and 5 HIAA concentrations were observed.

Bulbocapnine and the neuroleptic drugs have in common the property of inducing a cataleptic state in animals. Catalepsy ensues a few minutes after bulbocapnine administration (Loizzo et al 1971), while there is a delay of 1–2 h for neuroleptics (Boissier & Simon 1963; Timsit 1966). For neuroleptics an effect on the dopaminergic brain system has been described, consisting of a diminution of dopamine (DA) concentration (Falck et al 1969; O'Keeffe et al 1970; Honma & Fukushima 1976; Massotti 1977) and an increase of its acid metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Andén et al 1964: Bartholini et al 1975; Gessa & Tagliamonte 1975; Wiesel & Sedvall 1975; Wilk et al 1975; Massotti 1977; Westerink et al 1977) in the corpus striatum.

Only scattered data are reported on the effect of bulbocapnine on brain monoamines and their metabolites. The results of Sharman (1966) in the mouse indicate that the drug increases HVA concentrations in the striatum but has no influence on DA content. Analogous effects were observed by Sharman with chlorpromazine, haloperidol and spiroperidol.

We have examined the time-course of the behavioral and biochemical effects of a cataleptogenic dose of bulbocapnine in the rat, in particular the alterations of DA, DOPAC and HVA content in whole brain and in five separated areas. Concentrations of noradrenaline (NA), 5-hydroxytryptamine (5-HT), and 5-hydroxyindolacetic acid (5-HIAA) were also measured.

MATERIALS AND METHODS

Male albino rats of Wistar strain (Morini, Reggio Emilia), 120–160 g, were kept in a thermo-regulated room at 24 ± 2 °C, with free access to water and food. Bulbocapnine was dissolved in 0·01 M HCl, and adjusted to pH 6·0 by adding 0·1 M NaOH. The biochemical measurements were made 5, 15, 30, 60 and 120 min after intraperitoneal (i.p.) administration of 50 mg kg^{-1} of drug, in groups of at least five rats each. Catalepsy was evaluated at the same time intervals after administration in groups of 10 rats each.

The presence of a cataleptic state was assessed three ways: (1) the test of crossing the ipsilateral feet ('croisement des pattes homolatérales') according to Boissier & Simon (1963); (2) the Bouddha test according to Timsit (1966) (for these two tests the score was 1 if the animal accepted and maintained the imposed position for 60 s and 0 if it refused to accept the position); (3) the bar test. This consisted in putting the forelegs of the animal on a 10 cm high support and observing its reaction for 60 s. The scoring was: +2, if the rat remained immobile for 60 s; +1, if the rat moved slowly, without leaving the support; 0, if the rat withdrew its legs from the support.

Lesioned animals. Rats, 220–240 g, were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.); a bilateral electrolytic coagulation was stereotaxically placed in the substantia nigra or corpus striatum. The coordinates for corpus striatum (caudate-putamen) were obtained from De Groot's Atlas (1967); three lesions were placed according to the technique of Costall & Olley (1971a). For the coagulation of substantia nigra the coordinates were obtained from Costall & Olley (1971b). Current of 2.5 mA for corpus striatum and 0·5 mA for substantia nigra was passed twice for 7 s with a 10 s interval. The animals were then housed three per cage; sodium penicillin (50000 Units per day) was given for two days. Six days after the operation the animals were challenged with bulbocapnine. At the end of the experiment anatomical controls of the brain lesion were carried out. Sham-operated animals

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underwent the same surgical procedure except that no current was passed through electrode after insertion. At the end of the experiment anatomical controls were also carried out to exclude brain damage.

Biochemical measurements. The animals were decapitated and the brain immediately frozen in dry ice for the biochemical measurements in the whole brain. For measurements in the five areas, the brain was placed on ice and dissected in order: (1) hypothalamus; (2) corpus striatum, according to Glowinski & Iversen (1966); (3) limbic system, according to Bartholini (1976); (4) thalamus; (5) rest of the brain, including the greater part of cortex and hippocampus. The tissues were immediately frozen in dry ice.

Measurements of DA, DOPAC and NA were carried out as follows. The tissue was homogenized in n-butanol acidified to pH 2 with concentrated HCl, and centrifuged at 11 000 rev min^{-1}, 4°C, for 10 min. All the supernatant was transferred into a test tube containing a volume of 1 M Tris—0·1 M EDTA buffer pH 8·5, and a double volume of n-heptane. After this had been shaken and centrifuged the supernatant was transferred to a test tube containing 200 mg of activated alumina (neutral, grade I), washed twice with water, and 3 ml of 0·1 M HCl used to elute the three amines. Two aliquots of 0·5 ml were used to determine DA, according to Laverty & Taylor (1968), and NA, according to Maickel et al (1968). From the remaining 2 ml, the DOPAC was extracted with 4 ml of distilled ethylacetate which was re-extracted with 1·2 ml of a mixing solution (105 ml of water, 1·0 ml of 2M HCl, and 1·5 ml of distilled 1,2-diaminoethane) which was added. The fluorophore was activated according to the method of Murphy et al (1969). Blank values for DOPAC were obtained from the cerebellum.

Measurements of HVA, 5-HIAA, and 5-HT were made as follows. The tissue was homogenized in 0·1 M HCl. Zinc sulphate 10% to deproteinize, and 1 M NaOH to pH 7·0 were added. After centrifugation at 20000 rev min^{-1} +4 °C, for 10 min, 1 M HCl and 2% EDTA, and one and half volumes of distilled butyl acetate were added to the supernatant. After shaking and centrifugation, from the organic phase, 5-HIAA and HVA were extracted with 0·002 M Tris-HCl buffer pH 8·2. Two aliquots of Tris buffer were used to determine 5-HIAA, according to Curzon & Green (1970), and HVA, according to Andén et al (1963). The determination of 5-HT was carried out by buffering to pH 9·0 the residual aqueous phase, after butyl acetate shaking, with 15% sodium carbonate and borate buffer pH 10·0. 5-HT was determined according to Bogdanski et al (1956).

The fluorescence of biogenic amines was read on an Aminco-Bowmann spectrophotofluorimeter, after calibration. External and internal standards were used. The recovery was 88% for 5-HT, 85% for DA, 80% for DOPAC, 75% for NA and 5-HIAA, and 67% for HVA. All values were corrected for recovery. The data obtained in each group were compared to the control by the Multiple Comparison applied to the Analysis of Variance (Duncan test, Senter 1969).

RESULTS

Behavioural studies. The effects of bulbocapnine (50 mg kg^{-1} i.p.) on body posture are summarized in Table 1. The cataleptic state ensued 5 min after injection and lasted between 30 and 60 min. Of the three methods, the bar test and the test of 'croisement des pattes homolatérales' appeared to be more specific than the Bouddha test for revealing catalepsy. The onset of the immobility is preceded by jerks which also appear during the cataleptic state when the animals are handled. In rats with bilateral electrolytic coagulation of substantia nigra or corpus striatum, the drug-induced catalepsy in a smaller number of animals and was less intense. No differences were found between the two groups of lesioned animals (Table 2). The sham-operated animals presented the same scores of the intact rats.

Biochemical studies

Effects on the DA system. The time course of the effect of bulbocapnine on whole brain content of DA, DOPAC, and HVA is summarized in Fig. 1. A small but significant increase of DA content (124% of control) as well as a small decrease (89% of control) were found respectively 15 and 120 min after injection. A large increase of DOPAC (194% of

Table 1. Time course of effects of bulbocapnine (50 mg kg^{-1} i.p.) on body posture of rats. The fractions indicate the number of cataleptic responses over the total number of animals treated. At 0 time the rats had a score of 0/10 in all tests.

Time (min)	Bar test +2	Bar test +1	Crossed feet	Bouddha test
5	9/10	1/10	10/10	4/10
15	9/10	1/10	10/10	6/10
30	6/10	4/10	10/10	4/10
60	1/10	5/10	6/10	0/10
120	0/10	1/10	1/10	0/10

Table 2. Effects of bulbocapnine (50 mg kg^{-1} i.p.) on body posture after electrolytic coagulation of corpus striatum or substantia nigra in rats, 15 min after administration. The fractions indicate the number of cataleptic responses over the total number of animals treated.

	Bar test		Crossed feet	Bouddha test
	+2	+1		
Non lesioned	9/10	1/10	10/10	6/10
Lesions of substantia nigra	3/10	7/10	7/10	3/10
Lesions of corpus striatum	4/10	6/10	8/10	4/10

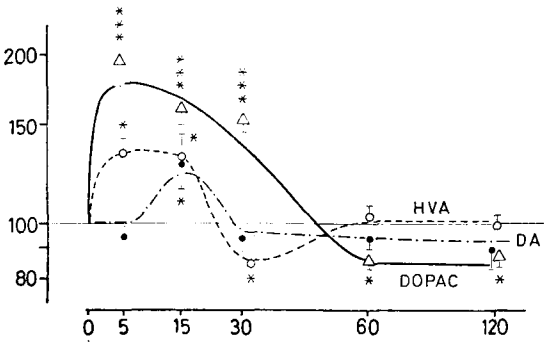

FIG. 1. The time courses of the changes in the concentrations of dopamine, 3,4-dihydroxyphenylacetic acid and homovanillic acid in the whole brain of the rat following the administration of bulbocapnine. The figure summarizes the data obtained at various times after administration of bulbocapnine 50 mg kg^{-1} i.p. At least 10 rats were used for each determination. Control values ($\mu g\ g^{-1}$) were as follows. DA, 0·950 ± 0·087; DOPAC, 0·124 ± 0·014; HVA, 0·152 ± 0·017. P values for the differences are shown above or below each point. None = not significant; * = $P<0·05$; ** = $P<0·01$; *** = $P<0·001$. Abscissa: log of time (min) after drug administration. Ordinate: log % modifications of content in DA, DOPAC, HVA.

control) was found 5 min after injection; this increase was reduced at 30 min, while at 60 and 120 min after bulbocapnine a small but significant decrease of DOPAC content was found (86% of control). A slight but significant HVA increase (135% of control) ensued in 5 min and lasted 15 min. A rebound phenomenon was registered 30 min after bulbocapnine, the HVA concentrations fell to 84% of control.

In the corpus striatum (Fig. 2A) a decrease of DA content (about 75% of control) was found 5 and 15 min after bulbocapnine. After 120 min the DA content returned to the normal values. The HVA increase (236% of control) ensued 5 min after treatment, while the DOPAC increase (196% of control) had a delay of 15 min. At the 60 min both values had returned to control.

In the limbic system (Fig. 2B) no modification of DA content was seen. A parallel increase of DOPAC (258% of control) and HVA (191% of control) was found 5 min after bulbocapnine and lasted up to 30 min. A rebound phenomenon was observed for DOPAC, since a decrease (68% of control) was observed 60 and 120 min after bulbocapnine.

In the rest of the brain (Fig. 2C), an increase of DA was observed 5 (152% of control) and 15 (132% of control) min after bulbocapnine. The DOPAC increase ensued 5 min (248% of control) and reached the maximum (402% of control) 15 min after bulbocapnine. The HVA increase reached the maximum (454% of control) 5 min after bulbocapnine. All values returned to normal at 120 min. No modifications of DA content was found in the hypothalamus and thalamus.

Effects on NA, 5-HT, and 5-HIAA. In whole brain, control values of NA were 0·420 ± 0·029 $\mu g\ g^{-1}$; after bulbocapnine a small but significant decrease (about 80% of control, $P<0·05$) ensued 5 min after treatment and lasted up to 120 min. A decrease of NA (about to 60% of control, $P<0·01$) was also found 5, 15 and 30 min after drug administration in the corpus striatum (control values: 0·815 ± 0·105 $\mu g\ g^{-1}$). No modifications of NA were observed in the other areas.

After drug treatment, no significant modifications of 5-HT and 5-HIAA were found in whole brain or in the separated brain areas, with the exception of the rest of the brain. In this area the control values of 5-HT and 5-HIAA were respectively 0·491 ± 0·061 and 0·051 ± 0·006 $\mu g\ g^{-1}$. After bulbocapnine, a marked increase of 5-HT (200% of control, $P<0·001$) was observed 5 min later and were still high at 15 min (168%, $P<0·01$) and 30 min (133%, $P<0·05$) after treatment, returning to normal at one hour. This was accompanied by a rise in 5-HIAA (144% of control, $P<0·01$) at 15 and 30 min.

DISCUSSION

The results indicate that bulbocapnine alters DA metabolism at the level of some discrete brain areas such as the corpus striatum, limbic system, and the rest of the brain, and it was less evident when the measurements were made in the whole brain.

These alterations are similar to those described for neuroleptic drugs of the phenothiazine and butyrophenone group. The mechanisms of acceleration of brain DA turnover induced by these drugs has been

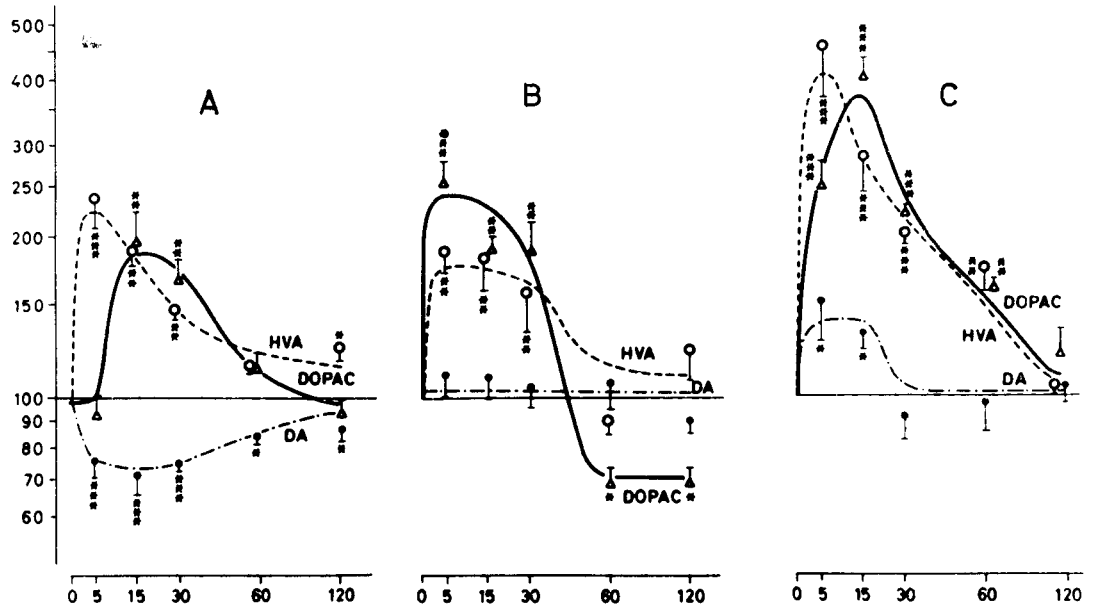

FIG. 2. The time courses of the changes in the concentrations of DA, DOPAC and HVA acid in regions of the brain of the rat given bulbocapnine. The figure summarizes the data obtained at various times after administration of 50 mg kg^{-1} i.p. bulbocapnine on DA (— · — · —), DOPAC (△——△), and HVA (○- - -○). At least 9 rats were used in each determination. Control values ($\mu g\ g^{-1}$) were as follows. Corpus striatum: DA, 7·87 ± 0·23; DOPAC, 1·25 ± 0·11; HVA, 0·51 ± 0·08. Limbic system: DA, 1·61 ± 0·08; DOPAC, 0·30 ± 0·06; HVA, 0·32 ± 0·08. Rest of the brain: DA, 0·26 ± 0·02; DOPAC, 0·033 ± 0·006; HVA, 0·024 ± 0·004. Statistical evaluation of the data was carried out as described in methods. *P* values for the differences are shown above or below each point. None = not significant; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$. Abscissa: log time (min) after drug administration. Ordinate: log % modifications of content in DA, DOPAC, and HVA. A, corpus striatum; B, limbic system; C, remainder of the brain.

studied extensively (Andén et al 1964; Carlsson 1975; Wiesel & Sedvall 1975; Wilk et al 1975; Zivkovic et al 1975; Bartholini 1976; Waldmeier & Maitre 1976; Scatton et al 1977). Present theories postulate that neuroleptics accelerate nerve impulse activity in DA neurons through a feedback mechanism in the nigro-striatal pathways triggered by block of DA receptors. It has also been suggested that neuroleptics block the DA-induced self-inhibition at the presynaptic level in the corpus striatum (see Di Chiara et al 1977). These mechanisms apply to other brain DA pathways since increase in DA acid metabolites has also been described for the nigro-limbic pathways (see Sedvall et al 1975). These alterations in DA metabolism have been linked both to the antipsychotic effect and to extrapyramidal disturbances induced by these drugs (Andén & Stock 1973).

The present results have indicated that bulbocapnine induces an elevation of DOPAC and HVA content in both striatum and limbic areas, as well as in the rest of the brain, which includes the greater part of the cortex and the hippocampus. In particular, the rise of HVA occurs immediately after administration in all areas and is parallel with the onset of the cataleptic state, thus suggesting a possible relation between these two phenomena. Time-course studies with neuroleptics show an increase in concentrations of DA acid metabolites 1–2 h after drug administration (Andén et al 1964; Wilk et al 1975), concomitant with the onset of the cataleptic state (Boissier & Simon 1963; Timsit 1966).

Bulbocapnine may induce the increase in HVA through a mechanism differing from that of neuroleptics. It could induce an immediate release of DA in the corpus striatum through a presynaptic effect, the catabolism of released DA leading to an increase in HVA. However, this hypothesis is not supported by the findings of Sharman (1966), who reported an increase in striatal HVA in mice treated with bulbocapnine. This increase was not present when the animals were pretreated with α-methyl-*p*-tyrosine (AMPT).

The increase in DA acid metabolites, found in the three areas considered, indicate that there is also an

increase in DA synthesis, as also reported for neuroleptics. The mechanism of this increase could be attributed to a block of DA receptors; however, in unpublished experiments we have found that bulbocapnine does not influence DA-stimulated adenylate cyclase activity in the corpus striatum. One explanation for this finding is that bulbocapnine acts on dopamine receptors which are not measured by the in vitro DA-sensitive adenylate cyclase system.

Other differences exist between bulbocapnine and neuroleptics such as haloperidol. Catalepsy induced by haloperidol is markedly reduced by destruction of the substantia nigra (Costall & Olley 1971b) or corpus striatum (Costall & Olley 1971a), while in the present experiments it has been found that such an intervention has only a slight influence on bulbocapnine catalepsy. On the other hand, haloperidol (Bariletto et al 1975; Gudelsky & Moore 1977) in the rat and spiroperidol (Sharman 1966) in mice induce a further fall in striatal DA content, lowered by previous treatment with AMPT. According to Sharman (1966) bulbocapnine in mice does not alter striatal DA and HVA concentrates lowered by AMPT, while spiroperidol induces an increase of HVA content.

Whatever the mechanism of action of bulbocapnine, the present results raise the question of the validity of the hypothesis connecting an effect of neuroleptic drugs on DA system with their antipsychotic activity. Our data point instead to a close link between extrapyramidal disturbances and alterations in DA metabolism.

We also investigated the possibility that NA or 5-HT might be involved in the mechanism of action of bulbocapnine and find that the drug's influence was slight on NA and 5-HT systems.

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