ferase partially purified according to Saelens et al.[7] and 1 µl (0.5 µCi) of S-adenosyl methionine methyl-^{3}H (sp. act. 10 Ci/mmole). The reaction was carried out at 20 °C for 10 min, and stopped by the addition of 65 µl of 0.7 M sodium borate buffer pH = 10.0. Then 900 µl of a mixture of toluene and isoamyl alcohol (3 v/2v) were added; after shaking and brief centrifugation, 600 µl of the organic phase was transferred to a scintillation vial and evaporated at 65 °C under a stream of air. The residue was redissolved in 1 ml of absolute ethanol; 10 ml of toluene containing 0.4% of diphenyloxazole and 0.01% of diphenyloxazolylbenzene were added and the radioactivity was measured by liquid scintillation.

As indicated in the table, the systolic blood pressure was higher in LH than in LN rats at the 3 different ages studied. The body weight was always higher in LH rats than in LN rats, the difference being significant only in 21-week-old animals.

In 5-week-old LH rats, the serum DBH activity was significantly higher (+28%, $p < 0.05$) than in age matched LN rats. This difference disappeared in 9- and 21-week-old rats, while in the both strains the serum DBH activity decreased with age (see table). In addition, a positive linear correlation ($r = 0.44$; $p < 0.05$, $n = 19$) was observed between the serum DBH activity and the systolic blood pressure of 5-week-old LH and LN rats.

Such an early increase in serum DBH activity has also been reported in young spontaneously hypertensive rats of the Japanese strain[8,9] and interpreted as reflecting an activation of the sympathetic nervous system. The same mechanism could be the origin of the increase in serum DBH activity observed in the 5-week-old hypertensive rats of our strain. Furthermore such an activation of the sympathetic nervous system is likely to be present in our rats since their enzymatic capacity to synthesize the peripheral catecholamines, as well as their urinary output of catecholamines, was increased[10-12].

In addition, it is interesting to note that serum DBH activity, as well as the other biochemical parameters related to the sympathetic nervous system[10-12] is only increased in 5-week-old LH rats, and returns to normal values in older animals, i.e. when the high blood pressure is fully developed. Such an evolution pattern, together with the existence in young LH rats of a positive correlation between the serum DBH activity and the systolic blood pressure values suggests that the sympathetic nervous system could be involved in the development of the genetically linked hypertension in that strain.

1 This work was supported by grants from DGRST (77.7.1930), CNRS (RCP 080.469, ATP A651-3358), INSERM (15.75.38) and the Fondation pour la Recherche Médicale Française.

2 To whom reprint requests should be addressed.

3 R.M. Weinshilboum, N.B. Thoa, D.G. Johnson, I.J. Kopin and J. Axelrod, Science *174*, 1349 (1971).
4 I.J. Kopin, S. Kaufman, H. Viveros, D. Jacobowitz, R. Lake, M.G. Ziegler, W. Lovenberg and F.K. Goodwin, Ann. intern. Med. *85*, 211 (1976).
5 J. Dupont, J.C. Dupont, A. Froment, H. Milon and M. Vincent, Biomedicine *19*, 36 (1973).
6 P.B. Molinoff, R.M. Weinshilboum and J. Axelrod, J. Pharmac. exp. Ther. *178*, 425 (1971).
7 J.K. Saelens, M.S. Schoen and G.B. Kovacsics, Biochem. Pharmac. *16*, 1043 (1967).
8 T. Nagatsu, T. Kato, Numata (Sudo), K. Ikuta, H. Umezawa, M. Matsuzaki and T. Takeuchi, Nature *251*, 630 (1974).
9 A. Nagaoka and W. Lovenberg, Life Sci. *19*, 29 (1976).
10 B. Renaud, S. Fourniere, L. Denoroy, M. Vincent, J.F. Pujol and J. Sassard, Brain Res. *159*, 149 (1978).
11 L. Denoroy, S. Fourniere, M. Vincent, B. Renaud, J.F. Pujol and J. Sassard, Brain Res. *162*, 184 (1979).
12 J. Sassard, B. Renaud, L. Denoroy, M. Vincent, S. Fourniere, L. Peyrin and J.F. Pujol, in: Nervous system and Hypertension, p. 234. Ed. P. Meyer and H. Schmitt. Wiley-Flammarion, New York - Paris 1979.

Effects of bicuculline and chlordiazepoxide on locomotor activity and avoidance performance in rats

V. Cuomo and I. Cortese

Institute of Pharmacology, University of Bari, I-70124 Bari (Italy), 17 December 1979

Summary. Bicuculline, at a dose of 1 mg/kg which, per se, failed to change locomotor activity in rats, counteracts the facilitating effect induced by chlordiazepoxide (10 mg/kg). Conversely, bicuculline (1 mg/kg) does not modify the decrease of motor activity and the disruption of avoidance performance induced by this benzodiazepine derivative (20 mg/kg).

Several reports seem to support the possibility that GABAergic mechanisms are involved in some behavioral effects of benzodiazepines. Particularly, it has been shown that GABA receptor blocking agents, such as picrotoxin and bicuculline, antagonize the effects of benzodiazepine derivatives on conflict schedules in rats[1,2]. Furthermore, picrotoxin has been shown to counteract diazepam-induced amnesic-like activity in rats[3].

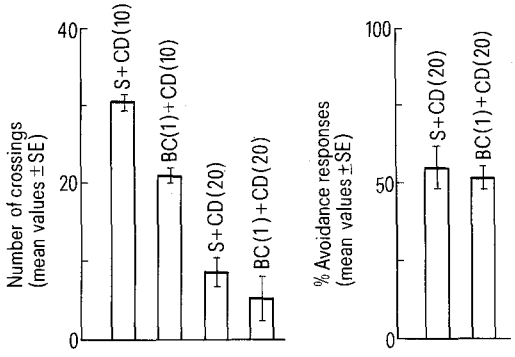
On the other hand, contradictory results have also been reported. Gardner et al.[4] found that the effects of GABA mimetic agents on locomotor activity in rodents differed considerably from those of benzodiazepine derivatives. Moreover, it has been shown that picrotoxin does not modify the depressant effect induced by diazepam on locomotor activity in rats and in mice[3,5]. Furthermore, Cook and Sepinwall[6] completely deny the possibility of an involvement of GABA in the anti-conflict activity of benzodiazepines. In the present experiment, bicuculline and chlordiazepoxide were employed in order to further evaluate the role of GABA in the effects of benzodiazepines on spontaneous locomotor activity and avoidance performance in rats.

Methods. Locomotor activity. The experiments were carried out on naive male Wistar rats weighing 240–260 g. The apparatus used in the present work was similar to that previously[7] and more recently[8] utilized for studying the spontaneous locomotor activity of mice. It consists of a series of toggle-floor boxes encased in a sound-attenuating chamber. Each box is divided into 2 compartments connected by an opening of 21 × 22 cm. For each rat, the number of crossings from 1 compartment to the other

Effects of chlordiazepoxide (CD) and bicuculline (BC) on locomotor activity (No. of crossings performed during a 10-min session) and avoidance performance (avoidance responses (%) performed in the drug session) in rats

Treatments (mg/kg)	No. of rats	No. of crossings (mean values ± SE)	Avoidance responses (%) (mean values ± SE)
Saline + saline	6	21.2 ± 2.74	89.2 ± 4.16
Saline + CD 5	6	22.3 ± 2.34	88.3 ± 2.78
Saline + CD 10	6	30.2 ± 0.94*	86.7 ± 3.57
Saline + CD 20	6	7.0 ± 2.55**	55.0 ± 6.70**
BC 0.5 + saline	6	23.3 ± 1.22	91.7 ± 2.47
BC 1 + saline	6	21.7 ± 2.45	88.3 ± 4.21
BC 2 + saline	6	13.2 ± 1.62*	84.2 ± 3.74

Significant changes with respect to control group (saline + saline): *$p < 0.05$; **$p < 0.01$.



Interaction between bicuculline (BC) and chlordiazepoxide (CD) on locomotor activity (No. of crossings performed during a 10-min session) and avoidance performance (avoidance responses (%) performed in the drug session) in rats. Data concerning the groups treated with saline (S) are the same as reported in the table; 6 rats were used in each group; mg/kg in parentheses. S+CD(10) vs BC(1)+CD(10): $p < 0.05$; S+CD(20) vs BC(1)+CD(20) not significant.

during a 10-min session was recorded by a microswitch connected to the tilting floor of the box.

Shuttle-box avoidance performance. The experiments were carried out on male Wistar rats weighing 250–265 g. The technique was that previously utilized for shuttle-box avoidance training of rats[9], guinea-pigs[10] and mice[11]. The apparatus consists of a series of shuttle-boxes encased in a sound-attenuating chamber. Each shuttle-box is divided into 2 compartments connected by an opening of 9 × 12 cm and operated by an electromechanical programming-recording unit. A light (3-W lamp) was alternately switched on in each compartment of the shuttle-box and used as conditioned stimulus (CS). The CS preceded by 10 sec the onset of the unconditioned stimulus (US) and overlapped it for 17 sec. The US was a scrambled foot-shock (50 V delivered through a 72.000 Ω resistance). By this procedure the light was present in the compartment for 27 sec (10 sec alone and 17 together with the US). In each session, the animal was subjected to 20 trials with 55-sec intertrial intervals. The interval between sessions was 24 h. An avoidance response (AR) was recorded when the animal avoided the US by crossing into the dark compartment within 10 sec after the onset of the CS. If animals failed to avoid the shock, they could escape it by crossing during the US (escape response).

In each session, escape responses were expressed as the percentage of the total number of trials in which animals failed to avoid the shock. Naive rats were subjected to daily conditioning sessions without any treatment. The day after completing a criterion of 80% or more AR in a session, the rats were allocated to the treatment groups. The groups were prepared so that they showed similar performance levels before the drug session.

In both studies, bicuculline (suspended in saline) and chlordiazepoxide (dissolved in saline) were given i.p. 15 and 10 min before the test session, respectively. The volume of all injections was 2 ml/kg.

Results and discussion. Locomotor activity. Concerning the effects of chlordiazepoxide and bicuculline (table), the analysis of variance for spontaneous crossings gave significant differences between groups ($F = 12.91$; $p < 0.001$). Moreover, a further analysis has been done in order to obtain individual between-groups comparisons, according to the Dunnett test[12].

Chlordiazepoxide, at the dose of 10 mg/kg, significantly increases the spontaneous locomotor activity of inexperienced rats. These results agree with the data reported by other authors in naive rats and mice[8,13,14]. Chlordiazepoxide, however, at higher dose levels (20 mg/kg) significantly decreases the number of crossings performed in the 10-min session. Locomotor activity impairments by benzodiazepines were previously described in rats and mice[5,15]. Bicuculline, at the dose levels of 0.5 and 1 mg/kg, does not influence the locomotor activity of naive rats. This agent, however, at the dose of 2 mg/kg, significantly reduces the number of crossings performed in the 10-min session. These last results complement those of Soubrie and Simon[3] showing that another GABA receptor blocking agent, picrotoxin, induces a decreased locomotor activity in rats. Bicuculline, at a dose of 1 mg/kg which per se failed to change motor activity in rats, counteracts the facilitating effects induced by chlordiazepoxide (figure). Conversely, bicuculline does not modify the decrement of motor activity induced by this benzodiazepine derivative (figure). The antagonism of chlordiazepoxide and bicuculline showed by this test suggests that a GABAergic system is involved in the facilitating effect of benzodiazepines on locomotion.

Shuttle-box avoidance performance. Concerning the effects of chlordiazepoxide and bicuculline (table), the analysis of variance for avoidance responses showed significant differences between groups ($F = 9.35$; $p < 0.001$). Individual between-groups comparisons were obtained utilizing the Dunnett test[12].

Our results do not show any significant effects on avoidance responses due to chlordiazepoxide at the doses of 5 and 10 mg/kg in comparison to the control group. As in the control group, escape responses following chlordiazepoxide (5–10 mg/kg) were always at a 100% level.

A significant disruption of avoidance performance by chlordiazepoxide was found at the dose of 20 mg/kg decreasing escape responses at a 58.16% level. Avoidance impairments by benzodiazepines have been described in rats and, in agreement with some authors, the inhibition of avoidance responses by benzodiazepine derivatives occurs at dose levels disrupting escape responding[16–18].

Bicuculline, at the doses tested in the present study, does not alter the animals' performance pattern, even though, at the dose of 2 mg/kg, a slight but not significant depression

of avoidance responding was observed. In the group treated with bicuculline, escape responses were always at a 100% level. Furthermore, our data show that bicuculline, at a dose counteracting the effects of diazepam on rats in a conflict situation (1 mg/kg)[2], does not antagonize the disruption of avoidance performance induced by chlordiazepoxide (20 mg/kg) in previously trained rats (figure). In this last group, escape responses were at a 52.50% level. Conversely, it has been shown that chlordiazepoxide counteracts the disruptive effects of strychnine, a glycine antagonist, on avoidance behavior in mice[11].

Whether a GABAergic system plays a role in determining the facilitating effects of chlordiazepoxide on avoidance behavior in rats is not yet known, and should be investigated.

1 L. Stein, C.D. Wise, J.D. Belluzzi, in: Mechanism of action of benzodiazepines, p.29. Ed. E. Costa and P. Greengard. Raven Press, New York 1975.
2 V.V. Zakusov, R.U. Ostrovskaya, S.N. Kozhechkin, V.V. Markovich, G.M. Molodavkin and T.A. Voronina, Arch. int. Pharmacodyn. *229*, 313 (1977).
3 P. Soubrie and P. Simon, Neuropharmacology *17*, 121 (1978).
4 C.R. Gardner, T.G. Johns and P. James, 7th int. Congr. Pharmac. 1978, abstracts, p.498.
5 P. Soubrie, P. Simon and J.R. Boissier, Neuropharmacology *15*, 773 (1976).
6 L. Cook and J. Sepinwall, in: Mechanism of action of benzodiazepines. Ed. E. Costa and P. Greengard. Raven Press, New York 1975.
7 A. Oliverio and C. Castellano, Psychopharmacologia *39*, 13 (1974).
8 M. Sansone, Psychopharmacology *59*, 157 (1978).
9 D. Bovet, G. Bignami and F. Robustelli, C. r. Acad. Sci., Paris, *266*, 778 (1963).
10 V. Cuomo and A. Marino, Pharm. Res. Commun. *6*, 531 (1974).
11 M. Sansone, Arch. int. Pharmacodyn. *215*, 190 (1975).
12 P. Dagnelie, in: Théorie et méthodes statistiques, vol.2, p.252. Ed. J. Duculot. Gembloux, 1970.
13 A.J. Christmas and D.R. Maxwell, Neuropharmacology *9*, 17 (1970).
14 A.S. Marriott and P.J. Spencer, Br. J. Pharmac. Chemother. *25*, 432 (1965).
15 L.O. Randall and W. Schallek, in: Psychopharmacology, a review of progress 1957–1967. Publ. Hlth Serv. Publ. *1863*, 153 (1968).
16 E.L. Abel, in: Drugs and Behavior, p.182. J. Wiley & So., New York 1974.
17 L. Cook and J. Sepinwall, in: Emotions, their parameters and measurement, p.379. Ed. L. Levi. Raven Press, New York 1975.
18 C. Morpurgo, Psychopharmacologia *8*, 91 (1965).

Oxytalan fibres in human dental pulp[1]

Ž. Bradamante, A. Pečina-Hrnčević and I. Ciglar

Department of Histology and Embryology, Faculty of Medicine, University of Zagreb, Šalata 3, P.O. Box 166, YU-41001 Zagreb (Yugoslavia), and Department for Pediatric Dentistry and Department of Dental Pathology, Faculty of Dentistry, University of Zagreb, Gunduličeva 5, YU-41001 Zagreb (Yugoslavia), 19 November 1979

Summary. Scarce and randomly oriented oxytalan fibres are present in the connective tissue of dental pulp in both deciduous and permanent teeth.

Oxytalan fibres are histochemically defined on the basis of their stainability with acid orcein, aldehyde fuchsin and resorcin fuchsin only after previous oxidation. The stainability is abolished by digestion with testicular hyaluronidase after oxidation, but not by digestion with elastase prior to oxidation. On the ultrastructural level, the oxytalan fibres appear as bundles of 150-Å-thick microfibrils[2,3]. Histochemically and ultrastructurally identical fibres represent the first step in the formation of mature elastic fibres ('pre-elastic fibres')[3,4]. The oxytalan fibre, elaunin and mature elastic fibres are therefore referred to as the elastic system fibres[5].

The oxytalan fibres, as a definite connective tissue component, are predominantly located in the periodontal membrane of man and some mammals, but they were also found in some other anatomical sites. Fullmer[6] found oxytalan-like fibres in the pulp of the developing deciduous tooth. However, a description of these fibres in the human dental pulp is lacking even in the comprehensive reviews[7]. In the present communication, we show that oxytalan fibres can be regarded as a regular tissue component of the human dental pulp.

Material and methods. Pulps from 5 deciduous (6–9 years) and 6 permanent (12–58 years) teeth were examined. Permanent teeth were extracted for orthodontic reasons or because of periodontal disease. The dental cavity was widely opened from both sides and teeth were fixed in toto in Lillie's buffered formol. After 3–5 days in fixative, the pulp was excochleated, dehydrated, embedded in parafin wax and sectioned serially at 7 µm. Deparafinized sections were examined after the following treatments[2,4]: Gomori's aldehyde fuchsin alone and after previous oxidation with peracetic acid, peracetic acid - aldehyde fuchsin - Halmi stain, PAS, Masson's trichrome stain, elastase - peracetic acid - aldehyde fuchsin, peracetic acid - testicular hyaluronidase - aldehyde fuchsin.

Results and discussion. No fibrillar structures in the pulp were stained with aldehyde fuchsin alone. The only exceptions were scarce elastic fibres around larger blood vessels. However, in sections stained with peracetic acid - aldehyde fuchsin without or with Halmi's counterstain, deep-purple, sharply outlined fibres were demonstrated in the intercellular matrix of the dental pulp of both deciduous and permanent teeth (figures 1 and 2). The interfibrillar matrix, as well as collagen fibres and nerves, stained very pale. In the central part of the pulp, the fibres were not oriented in any predominant direction but showed an apparent convergence to the walls of blood vessels. They were scarce in the central parts of the pulp and more numerous against its periphery. In some of the permanent tooth pulps, some wavy fibrils passed between odontoblasts. Fibres were not affected by pretreatment with elastase. However, they were not demonstrated in sections treated with testicular hyaluronidase between oxidation and staining with aldehyde fuchsin. The PAS staining also failed to demonstrate these fibres and only bundles of collagen fibres were demonstrated by the Masson's trichrome stain.

The results reported of histological staining show that fine