Na^+-independent binding of [^{3}H] muscimol to a membrane fraction of the brains of normal and dwarf mice

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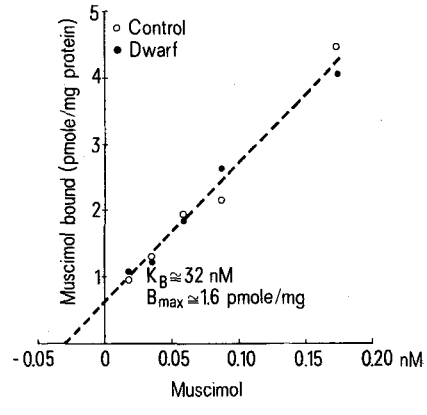
Summary. Although their body weights were decreased by about 77% and their brain weights by about 30%, high-affinity [^{3}H] muscimol binding to a cerebral membrane fraction was not altered in hereditary pituitary dwarf mice. Marked changes in the level of pituitary growth-associated hormones do not appear to be associated with a change in cerebral GABA-receptors.

Snell[3] discovered the hereditary dwarf mouse of the Black Silver strain. This mutation *(dw/dw)* is a recessive Mendelian character. Although adult dwarf mice weigh only about one-fourth to one-third as much as their normal littermates and have brains that are reduced by about 30% in weight, changes in the concentrations of many cerebral constituents do not occur or are not very dramatic[4,5]. In general, control of development of cerebral structures appears to be maintained, while the levels of synthesis of various cerebral constituents (e.g., gangliosides, phospholipids, cerebrosides, cholesterol, protein) are reduced in proportion to the decrease in brain weight[4,5]. Such developmental characteristics of the dwarf mouse appear to be caused by reduced levels of pituitary growth hormone[6-10] and thyroid-stimulating hormone[8,11].

At present, convincing evidence supports an involvement of dopamine, norepinephrine and possibly serotonin in the regulation of growth hormone[12] and an involvement of catecholamines and serotonin in the regulation of thyroid hormones[13]. Increasing evidence indicates that central GABA systems play roles in controlling the release of various hormones of the pituitary gland and hypothalamus; e.g. luteinizing hormone-releasing hormone (LHRH), adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (MSH), luteinizing hormone (LH), corticotropin releasing factor (CRF) and prolactin[14-19]. For these reasons, we studied the GABA-receptor in the brain of the dwarf mouse, which is known to be deficient in growth hormone, thyroid-stimulating hormone and prolactin[6-11]. In the present report, we compare the binding of [^{3}H]muscimol, a potent GABA-agonist[20], to membrane particles from dwarf and normal mice.

Homozygous hypopituitary dwarf mutants (*dw/dw*; dwarf) and control mice with normal phenotype (*dw/+* or *+/+*), all males, of the Black Silver strain were obtained from the Centre de Sélection et d'Elevages d'Animaux de Laboratoire (Orléans-La Source, France). No biochemical differences have been found between organs of *dw/+* and *+/+* animals[4,21]. Animals were fed a standard diet with water ad libitum, and were sacrificed at the age of 9-15 weeks.

The method of Zukin et al.[24] was used, with slight modification, to prepare a crude synaptic membrane fraction. Either 6 control whole brains or 8 homozygote dwarf brains were pooled and homogenized in 10 vols of 0.32 M sucrose solution at 0 °C; all further operations were conducted at 0 °C - 4 °C. Portions (20 ml) of homogenate were centrifuged at 1000 × g for 10 min. Supernatants were collected and centrifuged at 17,000 × g for 30 min. Resultant synaptosomal-mitochondrial (P_2) pellets were washed by resuspension in 20 ml of 0.32 M sucrose and recentrifugation at 17,000 × g for 30 min, and then resuspended in 11 ml of deionized water, combined in an Erlenmeyer flask, and sonicated with a Polytron Type PT 10-35 (half-maximal setting) for 30 sec. Portions of this preparation were distributed into tubes and centrifuged at 12,000 × g for 20 min. Resultant supernatants were collected, and pellets were rinsed with supernatant to collect their upper layers. Samples were centrifuged at 50,000 × g for 20 min, supernatants were discarded, and pellets were weighed and stored at −25 °C for 1-3 weeks.



Lineweaver-Burk plot of the [^{3}H]muscimol binding to crude synaptic membrane fractions of dwarf and control mice in Na^+-free, Tris-citrate medium (0 °C - 4 °C) represented as the binding that was sensitive to excess unlabelled GABA (specific binding). Values plotted represent the reciprocals of the differences between total binding (3 samples per point) and binding which occurred in the presence of 10^{-3} M unlabelled GABA (3 samples per point); values within each population varied less than 10%. A single line has been constructed. Binding (dissociation) constant (K_B; the concentration of [^{3}H]muscimol at which half-maximal binding occurred) and maximal binding capacity (B_{max}), determined by least-squares regression analyses, are indicated. Homozygous dwarf mutants *(dw/dw)* or control mice with normal phenotype (*dw/+* or *+/+*) were used.

Comparisons of brain and body weights and of some subcellular parameters in dwarf and control mice

Parameter	Control	Dwarf
Body weight (g)	30.1 ± 2.5 (40)	7.0 ± 1.2 (31)*
Brain weight (mg)	447 ± 14 (31)	311 ± 13 (24)*
Protein of pellet (mg/g)	71.8 ± 4.7 (30)	69.5 ± 6.3 (30)
Pellet weight (mg)	5.6 ± 0.3 (30)	3.8 ± 0.3 (30)*
[^{14}C]Sucrose distribution ratio[1]	0.76 ± 0.05 (30)	0.75 ± 0.06 (30)

Mean ± SD; numbers of samples in parentheses; * indicates $p < 0.001$ for comparisons between these values and their respective controls; Student's t-test (2-tailed). Homozygous dwarf mutants *(dw/dw)* or control mice with normal phenotype (*dw/+* or *+/+*) were used.

Frozen pellets were resuspended in 20 ml of deionized water, left at 22 °C for 20 min, and then centrifuged at 50,000 × g for 20 min; this cycle was repeated twice more, and the final pellets were weighed and resuspended in 3.2 ml of Na^+-free, Tris-citrate medium (50 mM; pH 7.1). Aliquots (100 µl) of tissue suspension plus 100 µl of Tris-citrate medium, either free of added substance or containing a final concentration of 10^{-3} M unlabelled GABA (to estimate 'specific' binding), were pipetted into small centrifuge tubes, mixed, and allowed to stand for 10 min. Then 250 µl of Tris-citrate medium containing 5.8–57.7 nM of [methylene-^{3}H(N)]-3-hydroxy-5-aminomethyl-isoxazole ([^{3}H] muscimol; 13.68 Ci/mmole and 62.6 - 626 nM of [^{14}C (U)] sucrose (final concentrations) were added. Samples were mixed, kept on ice for 20 min, and then centrifuged at 79,000 × g for 5 min to obtain final pellet and supernatant fractions. Radioactivity and protein were determined[22,23]. The [^{14}C]sucrose provided estimates of the amounts of supernatant fluid trapped in the pellets[22]. Radioactive products were purchased from New England Nuclear.

The table shows that the body weight of dwarf mice was only about 23% that of the controls and that their brain weight was only about 70% that of controls. Although pellet weights reflected the difference in brain weight, both the pellet protein content and the [^{14}C]sucrose pellet/supernatant distribution ratio did not differ between the two groups of mice (table). A Lineweaver-Burk plot of the 'specific' binding of [^{3}H]muscimol is shown in the figure. No difference existed between dwarf and control mice. Although their body and brain weights were markedly decreased, in comparison to control mice[4], cerebral membrane particles of dwarf mice bound [^{3}H]muscimol to a similar extent to those of controls. Preliminary experiments in our laboratory have revealed that the specific binding of [^{3}H]GABA also does not differ between dwarf and control mice. Hence, the marked decrease in brain weight that occurs under the abnormal hormonal control in dwarf mice does not appear to produce any change in the characteristics of high-affinity [^{3}H]muscimol (or [^{3}H]GABA) binding which appears to be associated with cerebral GABA-receptors.

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Arteriovenous anastomoses in the juxtamedullary cortex of human kidney

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Summary. In the juxtamedullary cortex of normal human kidney the existence of arteriovenous anastomotic channels is demonstrated by means of microscopic observation. A clear arteriovenous anastomosis occurring at the level of an afferent arteriole is also presented.

The existence of arteriovenous anastomoses in the normal human kidney is still disputed[1-3]. They have been postulated mostly on the basis of physiological experiments[4,5], but have never been shown by direct histological observation. By using a special technique of injection, Spanner[6] showed that arteriovenous anastomoses are always present in the renal capsule, cortex and sinus. On the other hand Clara[7], after reviewing the literature, suggested that only a patient histological study could objectively demonstrate the presence in the kidney of arteriovenous anastomoses, acting as regulating structures under physiological conditions. Aukland[3], referring to previous studies by other investigators, asserted that "... if arteriovenous shunts exist, they must be rare (or have diameters less than those of the afferent arterioles)".

Since the morphological foundation of arteriovenous shunts has not been clearly demonstrated in the human kidney, we examined samples of kidneys obtained from subjects in whom the case history and clinical-pathological study excluded renal damage.

Our research has been carried out on 10 cases post mortem: 6 men and 4 women, aged between 24 and 36 years, with