INDUCTION OF POSTURAL ASYMMETRY IN AN INTACT RECIPIENT BY BRAIN EXTRACT FROM A DONOR WITH THE SAME SYNDROME

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An attempt was made at the direct transfer of postural asymmetry, following removal of half the anterior lobe of the cerebellum in rats, to a recipient. Asymmetry of the hind limbs was shown to appear in spinal animals after injection of brain extract of pathological donors into their subdural space below the level of transection. The specificity of the resulting asymmetry depending on the side of injury to the donor's cerebellum was revealed. The asymmetry of the recipient completely copied the asymmetry of the donor. Brain extracts from control animals did not induce asymmetry. Additional confirmation was obtained of the polypeptide nature of the factor stimulating the development of postural asymmetry. Treatment of the extract with pronase deprived it of its activity.
KEY WORDS: postural asymmetry; brain extract; spinal cord; peptide factor.

Disturbance of spinal coordination following injury to the cerebellum has been familiar since the time of Luciani [10]. The long-term fixation of these disturbances by the spinal cord has received less study.

Work in Gerard's laboratory has shown that if the spinal cord in a rat with asymmetry of the hind limbs, caused by unilateral destruction of the anterior lobe of the cerebellum, is divided not less than 45 min after its onset, this asymmetry can be preserved in the animal for an indefinite time [5]. Investigations of the neurophysiological nature of the asymmetry thus arising has shown that it is based on enhancement of the myotatic reflex [3], possibly through increased activity of the interneuronal segmental apparatus [4]. The mechanisms of fixation of this unique and stable pathological state of the spinal centers have not yet been explained. It has been suggested that they resemble processes of memory consolidation, especially on the grounds that the time required for fixation can be lengthened with the aid of 8-azaguanine [6]. It was later shown that it can also be shortened, for example by administration of nicotine, strychnine [9], and ethimizole [2]. The participation of chemical factors in these mechanisms has also been demonstrated by the shortening of fixation of asymmetry in cerebellectomized recipient rats following intraperitoneal injection of brain extract from donor animals undergoing the same operation [8]. On the basis of the properties of the extracting substances it has been suggested that a factor of polypeptide nature accelerates the fixation of spinal asymmetry [7].

It remained to be discovered whether the active factor is in fact a polypeptide or whether it could be the oligonucleotide impurities present in the chloroform–methanol extract used by Daliers and Giurgea. Another question which remained unanswered was whether the donors' brain extract not only accelerates fixation of postural asymmetry in spinal recipients, but may also induce it in intact animals, for example, if the extract is injected directly into the subdural space corresponding to the lumbar segment of the spinal cord.

The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 350-400 g were used. Under pentobarbital anesthesia (40 mg/kg) half of the anterior lobe of the cerebellum was removed by suction. On recovery from the anesthetic the animal's hind limb on the ipsilateral side relative to injury was drawn up. This period was regarded as the time of onset of asymmetry. The whole brain of the donor together with the cerebellum was removed 17-20 h after its onset and kept at −17°C. The brain was thawed and homogenized in a mixture of chloroform and methanol (2 : 1), containing 1% concentrated HCl [8]. The homogenate was centrifuged at 10,000 g for 30 min, after which

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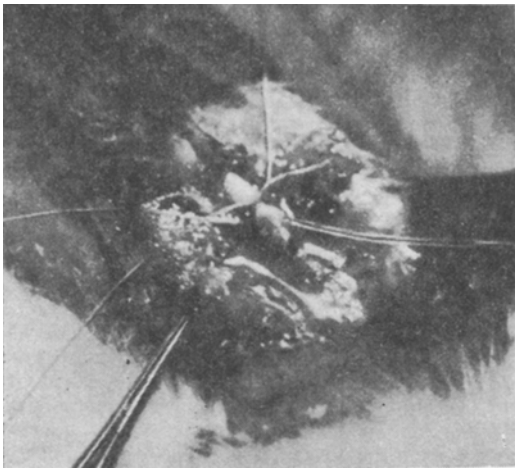

Fig. 1. Introduction of polyethylene cather into lumbosacral segment of spinal cord after transection.

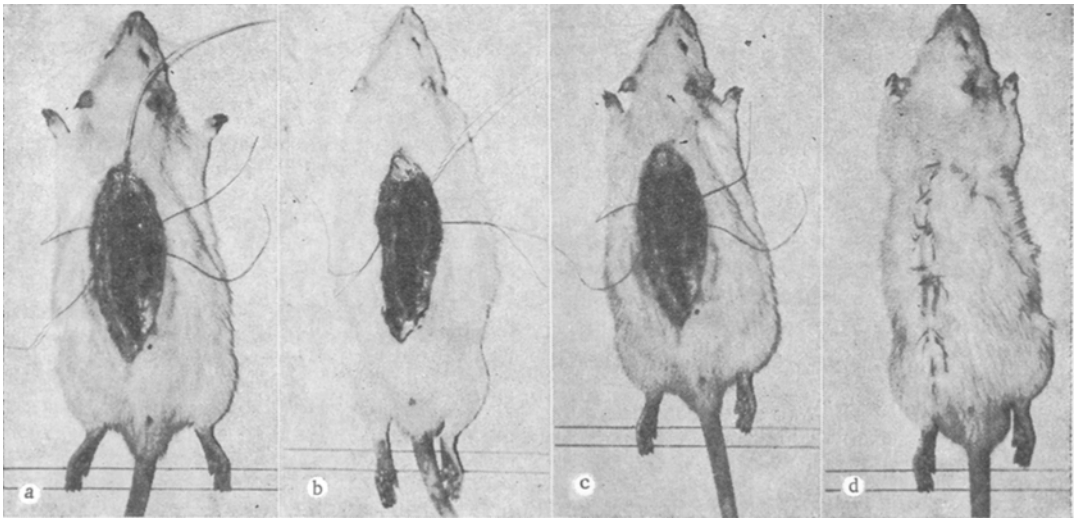

Fig. 2. Appearance of asymmetry in intact recipients during direct injection of brain extract of "right-sided" asymmetrical rats into subdural space of spinal cord: a) position of rat's limbs after end of movement of catheter; b) appearance of tonic convulsions of hind limbs during injection of brain extract; c) development of typical postural asymmetry after disappearance of convulsions; d) persistence of asymmetry next day.

the aqueous–methanol phase was separated and freeze-dried. The freeze-dried product was subjected to molecular-weight fractionation on a column with Sephadex G-25, after which the active fraction with a molecular weight of 1000 to 2000 daltons was collected [1]. To treat 100 mg of the active fraction 2 mg of pronase (from Serva, West Germany) was used. Hydrolysis was carried out for 10 h at 37°C in 0.1 M $NaHCO_3$, pH 8.3. The effectiveness of proteolysis was assessed by the disappearance of the active fraction and the appearance of a pool of free amino acids on fractionation of the digest on Sephadex G-25. In 55 rats under pentobarbital anesthesia laminectomy was performed in the region of thoracic segments T7-T9. The spinal cord was taken up accurately on a ligature and completely transected. Extract (15-20 mg) and control solutions (0.1 ml in volume) were injected subdurally into the distal portion of the transected spinal cord through a polyethylene catheter (Fig. 1). The onset or absence of asymmetry was recorded 15-60 min after injection of the extract. Asymmetry was regarded as significant it it amounted to 5 mm or more. In normal animals the natural asymmetry did not exceed 3 mm.

TABLE 1. Onset of Asymmetry in Spinal Animals after Subdural Injection of Brain Extract and Control Solutions

Substance	Total No. of recipients	Asymmetrical recipients	Activity of extract
Brain extract from right-sided donors	16	16 (right-sided asymmetry)	+
Brain extract from right-sided donors, treated with pronase	7	0	—
Brain extract from left-sided donors	8	8 (left-sided asymmetry)	+
Brain extract from control animals	7	0	—
Physiological saline	7	0	—
Ethimizole*	8	0	—

* Accelerates fixation of postural asymmetry in the same way as extract of pathological brain when given by intraperitoneal injection.

EXPERIMENTAL RESULTS

In the experiments of series I the active fraction of "right-sided" extract was injected into the animals. As the catheter was advanced to the lumbosacral segments of the spinal cord, small twitches of the hind limbs were sometimes observed, but these disappeared completely when movement of the catheter ceased. The animal's limbs remained symmetrical (Fig. 2a). Next, 0.1 ml of extract was slowly injected from a tuberculin syringe connected to the catheter. On injection of the extract the recipient rat developed tonic convulsions of the lower half of the body, which lasted for 10-15 min (Fig. 2b). The convulsions gradually diminished and disappeared, and flexion of the right hind limb with slight extension of the left developed (Fig. 2c). The amount of asymmetry varied from 5 to 12 mm and remained stable for many hours. Nor was it reduced the next day (Fig. 2d).

In the experiments of series II the active fraction was treated with pronase. The effectiveness of proteolysis was judged from the appearance of a pool of free amino acids and di- and tripeptides during passage of the fraction through a column with Sephadex G-25. The fraction when treated with pronase lost its activity: none of the seven rats into which it was injected developed asymmetry after the end of the convulsions. These observations point to a peptide nature of the factor causing postural asymmetry in the recipient rats.

In the experiments of series III, to test the specificity of action of the extract relative to the side of injury, brain extract from donors with injury to the anterior lobe of the cerebellum on the left side was injected into 8 rats without preliminary molecular-weight fractionation. All the animals developed left-sided flexion and right-sided extension of the hind limbs.

Series IV consisted of control experiments. Physiological saline was injected by the same method into seven animals. Neither convulsions nor asymmetry could be observed in any of the animals. Extract of intact brain was injected into another seven animals. All animals developed convulsions similar to those described above, but none of them developed asymmetry. Finally, eight rats received an injection of ethimizole in a dose of 3 mg/kg by the same rules, since previous observations showed that this shortened the time of fixation of postural asymmetry in the original model. In this case, just as when physiological saline was injected, neither convulsions nor asymmetry could be observed.

The results are summarized in Table 1.

A factor of peptide nature, specific with respect to the side of injury, thus participates in the formation of a stable pathological state of the spinal centers after injury to the cerebellum. The precise site of formation of this factor remains unknown, but what is certain is that 17-20 h after injury it accumulates in large quantities in the brain. Injection of this peptide factor into an intact spinal animal, into the subdural space of the spinal cord, causes persistent spinal asymmetry similar to that arising after injury to the cerebellum. This suggests that the peptide factor contains information on the character of the cerebellar injury and somehow induces changes in the recipient's spinal cord characteristic of this type of cerebellar lesion.

If the cerebellar model is found not to be unique and it can be shown that similar factors are produced in the presence of other brain lesions, a number of new problems requiring basically fresh approaches to their solution arise in the field of brain pathology and mechanisms of compensation.

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SOME PATHOGENETIC FACTORS IN EXPERIMENTAL "INDOMETHACINE" HYPERTENSION

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The concentrations of cyclic nucleotides (AMP and GMP) in the blood plasma, urine, and tissues, and morphological changes in the blood vessel walls of the kidneys were studied in rats with arterial hypertension induced by chronic inhibition of prostaglandin synthesis. Considerable thickening of the walls of the interlobular and arcuate arteries and marked constriction of the lumen, mainly on account of hypertrophy and swelling of smooth-muscle cells, were observed. Meanwhile the cyclic GMP concentration was increased, the cyclic AMP level lowered, and the cyclic AMP/cyclic GMP ratio reduced in the biological fluids. It is suggested that changes in the metabolism of the cyclic nucleotides are connected with organic and functional changes in the peripheral vascular system which lie at the basis of the increased general vascular resistance associated with arterial hypertension. KEY WORDS: indomethacine; prostaglandin; cyclic nucleotides; smooth-muscle cells; arterial hypertension.

In a paper published previously two forms of arterial hypertension (AH) were described in rats following administration of indomethacine, an inhibitor of prostaglandin (PG) synthesis, after unilateral nephrectomy or salt loading [1]. The antihypertensive effect of PG may be connected with their local action at the level of the peripheral vascular system, changes in which are characteristic of all forms of AH. It has been shown that PG counteracts the vasoconstrictor effect of the pressor hormones and blocks the secretion of catecholamines by nerve endings [6, 7].

The ultimate physiological action of PG in the tissue and, in particular, in the smooth-muscle cells of the blood vessels is mediated through a system of cyclic nucleotides [3]. It has been shown that cyclic AMP has a marked relaxant effect, and cyclic GMP a vasoconstrictor action [3, 5, 9]. It is also interesting to note that cyclic nucleotides have opposite effects on protein synthesis and on cell proliferation in certain tissues [2, 4].

The object of this investigation was to compare the cyclic AMP and GMP levels in biological fluids and tissues with the morphological state of the peripheral vessels in rats against the background of chronic inhibition of PG synthesis.

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