mosis with the veins which penetrate the psoas and cuadratus lumborum muscles and, further above, with the branches of the lower diaphragmatic vein.

B. Functional study. a) Clamping of the adrenal vein for 5 min. In this experiment, it is to be noted that, in spite of the total occlusion of the adrenal vein, no important lesions appeared in the adrenal parenchyme. There was a certain amount of congestion in the medullar sinuses and in the plexus of the ZR and ZFI, as well as in veins of the periadrenal fat and some zones of the subcapsular plexus. Some focal hemorrhages were also present at the union of the ZR and ZF.

b) Injection of methylene blue via renal artery. By means of this method, stained zones on the surface of the gland could be observed macroscopically. These zones, in general, corresponded to the trajectory of the capsular venules. But when all the periadrenal fat, except that lining the lower surface of the adrenal, was removed, this surface only appeared stained. The staining was more profuse and uniform in the animal injected intravenously via the systemic circulation. When examined microscopically, staining was observed in 7 of the 9 animals and was localized in the adrenal capsule and ZF, and less intensely and with little uniformity in other adrenal areas. In the case of the i.v. injection, focal staining was not observed in the parenchyme, but rather in the endothelium of the medullary capillaries and arterioles.

Discussion. Since the perirenal venous arch has multiple collaterals, one of its important properties would seem to be the possible existence of various drainage routes. The effectiveness of these collaterals is evidenced by their ability to substitue the clamped adrenal vein, whilst the adrenals suffer no important lesions, although hemorrhages do appear easily in these glands[12–14]. The capacity of this venous arch to drain renal and adrenal blood has already been verified in man and other species[1,5,7]. This vicariant action further implies one other important function. Blood from the kidney with a high concentration of angiotensin which is undiluted in systemic circulation can reach the adrenal gland via the venous arch and exert a strong stimulus on the ZG for the secretion of aldosterone. Similarly, blood from the adrenal carrying adrenaline, corticosteroids and aldosterone can also reach the kidney via this route.

12 L. M. Gonzalo-Sanz, Analyt. Anat. *14*, 285 (1965).
13 O. Fernández-Matías, Revta med. Univ. Navarra *16*, 323 (1972).
14 R. G. Harrison and M. J. Hoey, The adrenal circulation. Blackwell Scient. Publ., Oxford 1960.

A cholinergic modulator

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Summary. Soluble proteins obtained from presynaptic cholinergic vesicles have been tested regarding their effects to modify postsynaptic spike generation. The results suggest that these proteins (or derivatives, incl. glycopeptides) may act as modulators in increasing the effectiveness and duration of postsynaptic spike generation. They may partake in generation of homosynaptic (posttetanic) potentiation.

Homosynaptic (posttetanic) potentiation[1–7], the generation of a second postsynaptic spike of greater amplitude by 2 identical presynaptic shocks delivered within 1 sec, has been attributed to Ca^{2+}-transport related[8–10] presynaptic mechanisms[1,11–13]. In view of the recent discovery of protein-related specific neurotransmitter-modulators[14–17], it occurred to study whether presynaptic cholinergic vesicles contain cholinergic modulator(s). It seems that postsynaptic potentiation is, in part, inherent in the latency requirements of this modulator.

Methods. Soluble proteins were obtained from presynaptic cholinergic vesicles of adult rat brain and the electric

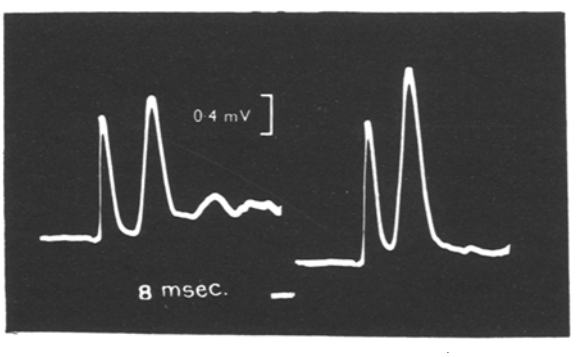

Fig. 1. Effects of protein extracts on homosynaptic potentiation. *X* 2 postsynaptic spikes generated by 2 supramaximal presynaptic pulses. The second spike had a larger amplitude than the first. *Z* Same presynaptic stimulation. Immediately after the first presynaptic pulse one of the vesicle extracts was added to the bathing fluid. The increased amplitude of the spike elicited by the second presynaptic pulse was significantly higher than that of the control. Horizontal mark: 8 msec; vertical mark: 0.4 mV.

1 M. G. Larrabee and D. W. Bronk, J. Neurophysiol. *10*, 139 (1947).
2 D. P. C. Lloyd, J. gen. Physiol. *33*, 147 (1950).
3 P. Nicolescu, M. Dolivo, C. Rouiller and C. Foroglou-Kerameus, J. cell. Biol. *29*, 267 (1966).
4 Y. Dunant, J. Physiol., Paris *59*, 17 (1967).
5 Y. Dunant, Prog. Brain Res. *31*, 131 (1969).
6 J. P. Tremblay, P. B. J. Woodson, W. T. Schlapfer and S. H. Barondes, Brain Res. *109*, 61 (1976).
7 P. B. J. Woodson, W. T. Schlapfer and S. H. Barondes, Neurosci. Abstr. *2*, part II, 999 (1976).
8 M. Kuno, Physiol. Rev. *51*, 647 (1971).
9 J. H. Pincus and S. H. Lee, Neurology *22*, 410 (1972).
10 R. S. Sohn and J. A. Ferrendelli, J. Pharmac. exp. Ther. *185*, 272 (1973).
11 J. C. Eccles and W. Rall, J. Neurophysiol. *14*, 352 (1951).
12 H. Gainer, J. P. Reuben and H. Grundfest, Comp. Biochem. Physiol. *20*, 877 (1967).
13 D. W. Esplin and B. Zablocka, Epilepsia *10*, 193 (1973).
14 E. Florey, Fed. Proc. *26*, 1164 (1967).
15 G. Zetler, in: Aspects of Neuroendocrinology, p. 210. Ed. W. Bargmann and B. Scharrer. Springer, Berlin 1970.
16 F. E. Bloom, B. J. Hoffer and R. A. Nicoll, Neurosci. Res. Bull. *10*, 213 (1972).
17 C. Torda, Naturwissenschaften *64*, 45 (1977); Pharmac. Res. Commun. *9*, 307 (1977).

Effect of various measures on postsynaptic spike generation in superior cervical ganglion of rat

Glucose content of bathing Krebs' solution	No. of experiments	Origin of protein extract	Nature of stimulation Procedure	Amount	Postsynaptic spikes Increase of amplitude of homosynaptic (post-tetanic)** potentiation (percent)	Length of potentiation (sec)
Glucose***	25	None	2 electric shocks	Supramaximal	15 ± 1.5*	1.1 ± 0.37
	25	Rat brain	2 electric shocks	Supramaximal	30 ± 3.2	5.5 ± 0.93
	12	Electric organ	2 electric shocks	Supramaximal	28 ± 2.4	4.7 ± 0.86
	10	Denatured	2 electric shocks	Supramaximal	18 ± 1.4	1.4 ± 0.7
						Length of postsynaptic response
No glucose***	20	None	Acetylcholine	0.05 µg/ml	13 ± 1.4	2 ± 0.9
	25	Rat brain	Acetylcholine	0.05 µg/ml	25 ± 2.6	24 ± 1.8
	12	Electric organ	Acetylcholine	0.05 µg/ml	22 ± 2.1	21 ± 2.1
	10	Rat brain	Acetylcholine	1 µg/ml	32 ± 2.8	111 ± 4.7
	10	Electric organ	Acetylcholine	1 µg/ml	27 ± 3.1	99 ± 5.1
	10	Denatured	Acetylcholine	0.05 µg/ml	16 ± 1.7	3 ± 1.3
		Glycopeptide:				
No glucose	10	None	Acetylcholine	1 µg/ml		2 ± 0.9
	10	0.5 µg/ml	Acetylcholine	1 µg/ml		15 ± 1.3
	10	1.0 µg/ml	Acetylcholine	1 µg/ml		22 ± 1.8
	10	1.5 µg/ml	Acetylcholine	1 µg/ml		28 ± 2.3
	10	Denatured	Acetylcholine	1 µg/ml		3 ± 1.2

* Mean ± SEM (differences generated by adding protein (or glycopeptide) extracts were statistically significant ($p < 0.001$), Student's t-test. ** Posttetanic potentiation (the name given by Larrabee and Bronk[1] for homosynaptic potentiation) in its usual meaning is generated by a series of stimuli and seems to result from the same 'modulator' process. *** Each preparation served as its own control. Control values were obtained by administration of acetylcholine alone. After having obtained 3 comparable mean values for amplitude and length of the postsynaptic train, acetylcholine was given in presence of one of the extracts.

organ of the Torpedo marmorata following the methods of Soller et al.[18] and Matsuda et al.[19]. The electric organ was used because its vesicles are almost exclusively cholinergic. Proteins were also obtained by DEAE-cellulose chromatography[20]. Protein content was ascertained following the method of Wannamacher et al.[21]. Glycopeptides were isolated from the protein preparation of Matsuda[19] following the method of Breckenridge and Morgan[22]. The method consisted essentially of pronase digestion, centrifugation and precipitation of the supernatant of cholinergic vesicle extracts by cetyl pyridium chloride. The supernatant was passed through a sephadex G-15 column to obtain a glycopeptide fraction. In order to ascertain that the preparation originated in the cholinergic vesicles, the acetylcholine content of the extracts was measured following the method of Goldberg and McCaman[23]. The acetylcholine content of rat brain extracts averaged 0.22 M and that of the electric organ 0.33 M. These values approximated the values obtained by other researchers[24,25]. The effects of vesicle extracts on postsynaptic spike generation were tested following the method of Dunant et al.[3–5]. Pre- and postsynaptic

18 M. Soller, H. Koenig, R. Mylroie, C. Hughes and C. Y. Lu, J. Neurochem. *21*, 557 (1973).
19 T. Matsuda, K. Saito, S. Katsuki, F. Hata and H. Yoshida, J. Neurochem. *18*, 713 (1971).
20 B. W. Moore and D. McGregor, J. biol. Chem. *240*, 1647 (1965).
21 R. W. Jr. Wannamacher, W. L. Bankes and W. H. Wunner, Analyt. Biochem. *11*, 320 (1965).
22 W. C. Breckenridge and I. C. Morgan, FEBS Lett. *22*, 253 (1972).
23 A. M. Goldberg and R. E. McCaman, J. Neurochem. *20*, 1 (1973).
24 A. M. Burton, in: Ganglioside Function, p. 123. Ed. G. Porcellani, B. Ceccarelli and G. Tettamanti. Plenum, N.Y. 1975.
25 V. P. Whittaker, W. B. Essman and G. H. C. Dowe, Biochem. J. *128*, 833 (1972).

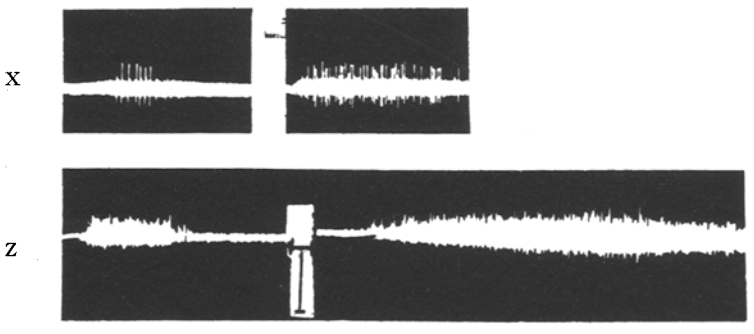

Fig. 2. Effects of protein extracts on length of postsynaptic train of spikes. Preparation was immersed in glucose-free Krebs' solution for 6 h. *X* Acetylcholine (0.05 µg/ml of bathing fluid). First record: control preparation: postsynaptic spike train. Second record: Protein extract was added to the bathing fluid before administration of acetylcholine. The same preparation served as its own control. Acetylcholine was added in 5-min-intervals. After having obtained 3 comparable postsynaptic response, protein extract (one of the extracts of vesicles) was added to the bathing fluid before adding acetylcholine. *Z* Acetylcholine (1 µg/ml of bathing fluid) was used as stimulus. First record: control (see *X*) 10-min rest periods. Second record: Vesicle extracts added before acetylcholine. Horizontal mark: 2 sec; vertical mark: 4 mV.

bioelectric processes were measured from the excised superior cervical ganglion of the rat, mounted on glass microelectrodes, by means of an oscilloscope (Tektronix, 561, 3A3). If immersed in Krebs' solution, the excised ganglion retained its bioelectric functions intact over 12 h. If immersed in glucose-free Krebs' solution, presynaptic functions and synaptic transmission vanished within 4 h, but postsynaptic spike generation remained intact for about 24 h. Presynaptic shocks of various intensities, generated by a transistor based stimulator were delivered across a stimulus isolation unit to presynaptic platinum microelectrodes at varying time intervals. Postsynaptic spikes were generated in preparations immersed in glucose-free Krebs' solution after inactivation of the presynaptic neurons, by acetylcholine (0.01–1 µg/ml of bathing solution). All preparations were presensitized with physostigmine salicylate (5 µg/ml bathing solution). Statistical significance of the observed differences of correlation coefficients were ascertained by Student's t-test.

Results. 2 supramaximal presynaptic shocks, delivered at 50-msec-intervals, generated maximum homosynaptic potentiation. The length of potentiation exceeded, at times 1 sec. Protein (peptides, glycopeptides) extracts of cholinergic vesicle contents of either the rat brain or the electric organ increased the amplitude and the length of potentiation (table, figure 1). In preparation with inactivated presynaptic neurons, these extracts (added to the bathing fluid) increased the amplitude of postsynaptic spikes (generated by acetylcholine), and significantly increased the length of the generated postsynaptic spike trains in a dose-dependent manner (table, figure 2). Heat denatured extracts lost their ability to affect postsynaptic spike generation by both endogenous (presynaptic stimulation) and exogenous acetylcholine. The latency of the first postsynaptic spike was about 10 msec shorter than the onset of potentiation by the extracts. In the concentration used, the extracts alone did not initiate postsynaptic spikes.

Discussion. Presynaptic cholinergic vesicles contained an agent that possessed the minimum necessary properties of a 'modulator'[13] of acetylcholine effects, e.g.: 1. In absence of acetylcholine the extract did not generate postsynaptic spiking. 2. The extract potentiated the effect of both exogenous and endogenous (released during presynaptic stimulation of cholinergic neurons) acetylcholine. 3. Postsynaptic effects outlasted presynaptic stimulation (release of acetylcholine). 4. Postsynaptic cholinergic effects were smoothened (spike amplitude decreased gradually). 5. This madulator effect was dose-dependent. 6. The modulator was either a protein or its derivative (peptides, glycopeptides).

The modulator effect seemed to be multidetermined, e.g.: 1. Adsorption on this protein may facilitate transsynaptic acetylcholine transport, and may delay its hydrolysis. 2. Gradual release may smoothen the postsynaptic effects of acetylcholine. 3. Since the latency of the onset of modulation exceeded the latency of the first postsynaptic spike, postsynaptic processes seemed also to contribute. 4. Because of Ca^{2+}-transport proteins also occur in the vesicle content[26], promotion of transsynaptic Ca^{2+}-transport may facilitate the effects of Ca^{2+} on pre- and postsynaptic de- and repolarization[27]. It has not yet been ascertained which of the 22 known proteins contained in cholinergic vesicles are responsible for the modulator effect. Soller et al.[18] suggested that the acidic lipoproteins are carriers binding the putative neurotransmitter. Transport properties were also attributed to glycoproteins[22]. Even though glycoproteins prevail in membranes, glycopeptides may also occur in the vesicle fluid[22]. During presynaptic stimulation cholinergic vesicle walls is believed to fuse with the terminal membrane of the presynaptic neuron, and verse part of their content into the synaptic cleft. Musick and Hubbard[28] did collect proteins from the effluent of stimulated mouse phrenic nerve. In addition to acetylcholine, the fluid ejected from these vesicles contained proteins (or their derivatives [peptides, glycopeptides]) with modulator effects. Generation of homosynaptic (posttetanic) potentiation is inherent in the different latency-requirements of acetylcholine and the modulator to initiate postsynaptic effects. The longer latency of the onset of modulator effect prevents the modulator from changing the first (or early) postsynaptic spikes.

26 G. Prestipino, D. Ceccarelli, F. Conti and E. Carafoli, FEBS Lett. *45*, 99 (1974).
27 C. Torda, Experientia *30*, 1154 (1974); Int. Rev. Neurobiol. *16*, 1 (1974).
28 J. Musick and J. I. Hubbard, Nature *237*, 279 (1972).

Monosynaptic inhibition of thalamic neurons produced by stimulation of the substantia nigra

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Summary. Electrical stimulation to the substantia nigra (Pars reticulata) produced a monosynaptic inhibition of the neurons of the ventromedial nucleus of the thalamus in anesthetized cats.

One of the major outputs of the basal ganglia is the pallidothalamic pathway. Uno and Yoshida[2] have demonstrated that neurons in the rostroventral portion of the thalamic nucleus ventralis lateralis (VL) receive monosynaptic inhibition from the pallidal nucleus of the cat. Nigro-thalamic fibres form another output system from the basal ganglia[3–8]. In this paper we show that stimulation of the substantia nigra monosynaptically produces IPSPs (inhibitory postsynaptic potentials) in neurons of the ventromedial nucleus (VM) of the thalamus.

Materials and methods. Cats were anesthetized with sodium pentobarbital (30 mg/kg). Platinum-iridium needles of 0.2 mm diameter, insulated except for the very tip, were used as stimulating electrodes. 6 needles were combined and inserted stereotaxically into the SN[9]. Stimulating pulses were applied between 2 neighboring SN-