Zusammenfassung. Die Wirkung von Glutamat wurde auf das Membranpotential von Rückenmarkneuronen des Menschen und der Ratte in Gewebekultur untersucht. Entfernung der Natriumionen aus der extrazellulären Flüssigkeit führt zu einem Verschwinden der durch Glutamat erzeugten Depolarisation. Diese Befunde weisen darauf hin, dass Glutamat, welches eine vermutliche Überträgersubstanz im Rückenmark ist, die Permeabilität der Neuronenmembran für Natriumionen erhöht. Die Versuche zeigen ferner, dass die Gewebekultur ein ausgezeichnetes Modell ist zur Abklärung von ionalen Mechanismen, welche der Wirkung von Überträgersubstanzen im Zentralnervensystem zugrunde liegen.

L. Hösli, P. F. Andrès and Elisabeth Hösli

Department of Neurophysiology, Neurological Clinic of the University of Basel, Socinstrasse 55, CH–4051 Basel (Switzerland),
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Decrease in the Sympatho-Inhibitory Action of Clonidine after Destruction of the Sympatho-Inhibitory Area

There is considerable evidence for a centrally mediated decrease in the sympathetic tone by clonidine[1–6]. The main site of action has been localized in the medulla oblongata by transection experiments[7,8] and injection into the cephalic arteries [3,9,11]. Piperoxan [10,11] and yohimbine[11] antagonized this effect. Therefore an activation of central α adrenoreceptors has been proposed[10,11] as the cause of the sympatho-inhibitory effect. A noradrenergic mechanism inhibiting the sympathetic tone has been suggested to be localized in the medulla oblongata[10,11].

Clonidine has a much less reducing effect on the increase in the sympathetic tone brought about by central stimulation than it has on the spontaneous sympathetic tone[5,12]. Clonidine reduced the effect of submaximal stimulations but did not change or even increase the effects of supra-maximal stimulations. Waite[12] hypothesized a summation of the effects of clonidine on central mechanisms with the influence of the baroreceptors fibres on central sympathetic tone. At submaximal stimulation, the influences coming from the baroreceptor pathway are also submaximal and their sympatho-inhibitory effect could summate with the effects of small doses of clonidine. For supramaximal stimulations, the sympatho-inhibitory mechanisms are maximally activated and a summation is not possible. Therefore the increase in the sympathetic tone induced by central stimulations is unopposed. On the other hand, the first synapse of the baroreceptor pathway was localized into the nucleus tractus solitarii[13,14] and the nucleus reticularis paramedialis[15], two regions where noradrenaline-containing neurons were found[16]. A noradrenergic link in this pathway was therefore proposed[10,11].

In these hypotheses, clonidine was suggested to mimic or to activate sympathoinhibitory mechanisms in the medulla oblongata. To check this supposition, destruction in the bulbar depressor area were performed and their possible influence on the sympatho-inhibitory effect of clonidine was investigated.

Methods. Cats of either sex weighing 2–3.5 kg were anaesthetized i.v. with a mixture of chloralose (0.050 g/kg) and urethane (0.250 g/kg). They were tracheotomized but allowed to breathe spontaneously. Carotid blood pressure was recorded by means of a Statham P 23 Db pressure transducer on a San'Ei Visigraph and on a beam of a cathodic oscilloscope Tektronix 502 A using the D.C. channel.

The splanchnic nerve was isolated by a paravertebral incision retroperitoneally at its exit of the diaphragm. The nerve was stripped of its sheath and a bundle of fibres was placed on a pair of platinum electrodes. The nerve was cut distally. The splanchnic discharges were amplified by a Tektronix 122 preamplifier using the frequencies between 80–1000 Hz. The discharges were exposed on the second beam of the oscilloscope. The discharges were picked up at the output of the preamplifier, fully rectified and smoothed by means of an operational amplifier using a R. C. network of a 0.2 second time constant. The smoothed discharges were exposed on a channel of the Visigraph. The amplitude of the smoothed signal was approximately proportional to the amplitude and frequencies of the input potential. The value of the smoothed signal before the administration of clonidine was taken as 100% and the zero was determined after the death of the animal.

To expose the medulla oblongata, the head of the animal was placed in a stereotaxic instrument (La Précision Cinématographique Française); the muscles of the neck were incised and reclined. The occipito-atlantoïd membrane was opened and a part of the occipital bone was removed in order to expose the floor of the 4th ventricle. The cerebellum was gently reclined. A stainless steel electrode of 0.3 mm in diameter, varnished on its whole length except for 0.5 mm at its tip,

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was placed on the floor of the 4th ventricle 1–3 mm rostral to the obex in the midline. An area where electrical stimulation (1–3 volts, 3–30 Hz, 2 msec) induced hypotension and bradycardia was chosen. The destruction was performed with a high frequency current of 10 mA applied during 45 sec. The electrode was displaced 2 mm rostrally, 2 mm caudally, and 2 mm in depth. The destruction was taken as satisfactory when pulling on a carotid artery did not induce a decrease in blood pressure or heart rate.

Similar experiments were performed on dogs of either sex, weighing 5–8 kg and anaesthetized with chloralose (0.100 g/kg i.v.).

Results. Clonidine (10 µg/kg i.v.) induced, in 7 sham operated cats, the usual changes: a hypertension followed by a long-lasting decrease in blood pressure and a bradycardia. Mean blood pressure decreased from 120 ± 6 mm Hg (144 ± 6/108 ±4) before clonidine to 95 ± 4 mm Hg (117 ± 5/81 ± 4) 20 min after the drug. The difference was highly significant ($P < 0.01$). Splanchnic discharges were strongly reduced (70 ± 8%).

After a large destruction of the depressor area, initial blood pressure was significantly lower than in sham operated cats and was 97 ± 9 mm Hg (121 ± 9/72 ± 10). Clonidine induced an increase in blood pressure but the bradycardia and the secondary decrease in blood pressure did not appear; 20 min after the administration of clonidine, blood pressure was 98 ± 8 mm Hg (119 ± 7/80 ±8) which was not significantly different from the initial value. Splanchnic discharges were reduced only by 25 ± 6%.

In dogs it was easier to perform the destruction of the depressor area without changing blood pressure. Figure 1 shows the record of a dog after a large destruction of the medullary depressor area. In the upper row, clonidine (10 µg/kg) induced a rise in blood pressure followed by a decrease and heart rate was slowed; splanchnic discharges were reduced to 50%. 2 h later the action of clonidine had disappeared, a larger destruction was performed and clonidine induced only a small rise in blood pressure but no bradycardia. The decrease in splanchnic discharges was only 20%.

Figure 2 shows the dose response curve for the hypotensive effect of clonidine administered in small doses (0.3–1 µg/kg) into the vertebral artery of a dog. After recovery, a large destruction in the depressor bulbar area was performed and the dose response curve of clonidine was shifted to the right, indicating a decreased effectiveness.

Discussion. The present work was undertaken to discover the influence of destruction of the bulbar depressor area on the sympatho-inhibitory effect of clonidine. The results indicate a reduced effectiveness of clonidine after the destruction.

In cats, the fall in blood pressure was abolished. However, this result cannot be taken as evidence for a decreased action of clonidine at central sites of action. In fact, in cats with the depressor area destroyed, the initial blood pressure was lower than in control animals and was not significantly different from blood pressure on control animals measured 20 min after the administration of clonidine. After the destruction, splanchnic discharges were also reduced at a similar extent than after clonidine in control animals. It could therefore be concluded that the destruction reduced the sympathetic tone to the same extent as clonidine. This result was probably due to the fact that pressor and depressor areas are intermingled[17,18]. However, that clonidine did not reduce the splanchnic discharges further indicates the medulla oblongata as the main site of action of the drug.

In dogs the destruction of the sympatho-inhibiting area was easier to perform, and after this destruction the sympatho-inhibitory effect of clonidine was weaker. The dose response curve for the hypotensive effect of clonidine administered into the vertebral artery was shifted to the right, indicating a decreased sensitivity.

Therefore, these experiments indicate that the main site for the hypotensive effect of clonidine is localized in the depressor medullary area lying at the level of the obex. They afford some support for the hypothesis that clonidine mimics or activates central sympatho-inhibitory mechanisms. However, a large destruction was necessary to decrease the sympatho-inhibitory effect of clonidine, and a definite conclusion as to the precise site of action was not possible, since the reduction in the sympathetic discharges induced by clonidine was never completely abolished in dogs, therefore

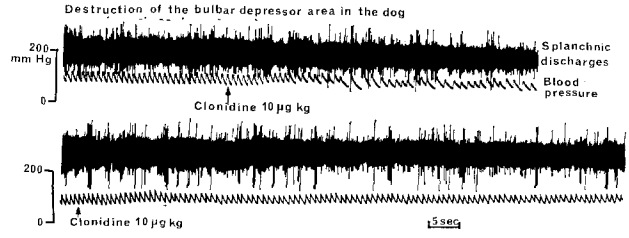

Fig. 1. Decrease in the sympatho-inhibitory action of clonidine after a destruction of the bulbar area in the dog. The upper row shows the classical effects of clonidine: a rise in blood pressure followed by a decrease (not shown), a bradycardia and a reduction in splanchnic discharges. The lower row was taken 2 h after the recovery. A large destruction was performed in the depressor medullary area. Clonidine in the same dosage induced a rise in blood pressure, did not slow the heart rate and induced a much lesser decrease in splanchnic discharge than before the intervention.

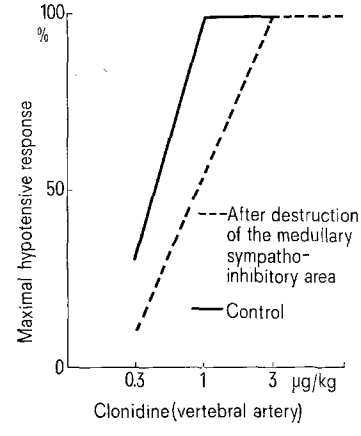

Fig. 2. Decrease in the hypotensive effect of clonidine injected into the vertebral artery of a dog after a large destruction of the bulbar sympatho-inhibitory area. The figure shows the dose response curve of the hypotensive effect of clonidine injected into the vertebral artery of a dog (—). 2 h after the recovery, a large destruction was performed in the bulbar depressor area: the dose-response curve (---) was shifted to the right, but the maximal effect was not changed.

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suggesting other sites of action, possibly the spinal cord. Further work has to be performed to localize this effect.

Résumé. La destruction extensive de l'aire dépressive bulbaire provoque chez le chat une chute de la pression artérielle, du rythme cardiaque et une diminution de l'activité électrique du nerf splanchnique. La clonidine ne provoque alors qu'une augmentation de la pression artérielle, sans hypotension secondaire, ni diminution des potentiels splanchniques. La perte des influences sympatho-inhibitrices de la clonidine est encore plus nette chez le chien, car la destruction extensive de cette aire ne provoque qu'une diminution légère de la pression artérielle et de l'activité électrique du nerf splanchnique.

H. Schmitt, Hélène Schmitt and Simone Fénard

Faculté de Médecine Paris, Broussais Hôtel Dieu
15, rue de l'Ecole de Médecine
F–75 Paris 6ème (France), 30 April 1973.

Choline Activation of Lithium Transport

The therapeutic usage of lithium to treat manic depressives is well established[1–14]. Lithium carbonate is currently accepted as the standard treatment and typically is administered in daily amounts of from 1.5 to 3.6 g total. The dosages are usually given thrice daily[6] in 500 to 1200 mg amounts and result in a serum concentration of from 0.6 to 1.5 meq lithium/l.

While the general response to lithium treatment has been positive, a number of side effects have been reported. These include essential tremor, anorexia, vomiting and diarrhea[6]. Investigators have reported dermatitis[7], increases in thyroid size and iodide metabolism[8–11], EKG changes[6], and an increase in intracellular and extracellular water content[12]. Several reports[13,14] indicate that propanolol (Inderal) may be used to treat essential tremor unless the lithium is given concurrently with tricyclic anti-depressants[14]. Propanolol is known to inhibit cholinesterase activity and is used with this in mind.

In view of the aforementioned, it would seem beneficial to devise a system in which lithium transport was accelerated with the possibility of achieving similar therapeutic effects through lower dosage levels. Such a system is one in which choline facilitates the initial flow of lithium across bovine erythrocyte membranes as reported in this preliminary communication, the full details of which will be published elsewhere[15].

Material and method. Heparinized bovine blood, obtained by jugular puncture, was collected by centrifugation, washed twice with Normal Ringers buffer, and resuspended in 9 ml of buffer containing dextrose (0.2%) and choline iodide where appropriate. Suspensions were incubated at 37°C and 1 ml of lithium sulfate was added to initiate the reaction. At convenient intervals, 1 ml samples were withdrawn, centrifuged, and the supernatant separated. The pellets were treated with 1 ml of hemolyzing solution[16]. The resulting solution and the supernatant were analyzed separately for lithium content as before[16]. Efflux measurements were also performed as reported previously.

Results and discussion. A number of influx and efflux studies were completed at 37°C as a function of hematocrit. The data was clearly first order within a period of 30 min or less. The pseudo first-order rate constants were independent of hematocrit with k_e (efflux, 0.045 $\pm$ 0.001 min^{-1}) greater than k_i (influx, 0.035 $\pm$ 0.004 min^{-1}).

A cell volume study[15] in which choline was seen to shrink the average size of a bovine erythrocyte led to an investigation of the effect of choline on lithium influx, in which the total concentration of lithium and choline was kept at 4 mM in the initial supernatant. The influx rate constant increased from 0.052 $\pm$ to 0.064 $\pm$ 0.008 as the choline concentration was increased from 0.8 to 3.2 meq/l. When the lithium concentration is held constant (4 mM) and the choline concentration is increased from 4 to 12 mM, the value of k_i increased from 0.043 $\pm$ 0.005 to 0.075 $\pm$ 0.010. Specifically, $\log_e k_i = -3.37(\pm\ 0.07) + 0.068(\pm\ 0.008)$ [choline], where $\pm$ values indicate standard deviations. In addition, k_i was independent of the hematocrit (0.13–0.32) for a given choline concentration.

Previous workers[17–19] have studied the transport of choline across erythrocyte membranes. Askari[17] has compared choline uptake with potassium by means of Michaelis kinetics. His results include a larger V for potassium and a smaller K_m for choline which were interpreted as indicating fewer binding sites for choline and a higher site affinity for choline.

Martin[18] noted that choline transport obeyed first-order kinetics for periods of 10 min or more at low hematocrit values (0.02–0.03). He also established that there is no apparent adsorption of choline to the cell membrane and that ouabain (5×10^{-5} M) had no effect on choline transport. Using the data from Table I and Figure 7 from[18] it is possible to calculate values for k_e (0.035) and k_i (0.023) in min^{-1} for choline.

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