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DUBRAVKA POTKONJAK

THE RELATIONSHIP BETWEEN RIGIDITY AND CHANGE IN WHOLE BRAIN ACETYLCHOLINE CONCENTRATION PRODUCED BY INJECTION OF FLUPHENAZINE IN THE RAT

Arvidsson et al. (1966) advanced the hypothezis that drugs inducing a shift of the monoaminergic-cholinergic equilibrium in favour of the cholinergic neurons in the brain of experimental animals — were able to induce a state of rigidity in these animals. This rigidity manifested itself by an increased resistance against flexion of the limbs and by a reduction of gamma and an increase of alpha motoneurone response. Because of this that rigidity was said to be of an alpha type (Steg, 1964; Roos and Steg, 1964; Arvidsson et al., 1966, Jurna et al., 1969). According to Arvidsson et al. (1966 and 1967) chemically different drugs could induce the mentioned shift either facilitating cholinergic transmission (physostigmine) or inhibiting monoaminergic transmission (reserpine, chlorpromazine, phenoxybensamine, haloperidol). If the balance is restored by drugs which either stimulate monoaminergic function (L-DOPA) or depress cholinergic function (atropine) — rigidity is eliminated.

Reserpine and phenothiazine derivatives, which have been reported to produce rigidity in animals as well as in man (Glow, 1959; Courvoisier et al., 1957; Steg, 1964) — are generally believed to induce rigidity in rats by an inhibition of monoaminergic transmission (Roos and Steg, 1964; Arvidsson et al., 1966 and 1967; Hornykiewicz, 1966; Jurna et al., 1969) either depleting the stores of the transmitter substances (reserpine) or preventing the transmitter to act at the receptor site (phenothiazine derivatives). Reserpine, however, has been reported not only to inhibit monoaminergic transmission, but to affect cholinergic transmission as well. This drug has been shown to produce a significant increase in the acetylcholine content of different areas of the dog's brain (Malhotra and Pundlik, 1959), of the rat brain stem (Malpica et al., 1970) and of the whole rat brain (Giarman and Pepeu, 1962).

The aim of this work was to investigate whether the phenothiazine derivative fluphenazine, apart from inhibiting monoaminergic transmission, can, like reserpine, affect cholinergic transmission also. In order to examine this, the relationship between rigidity and brain acetyclholine

concentration after fluphenazine administration into rats was studied. In an attempt to provide more information on this new mode of action of fluphenazine as a rigidity — producing drug — it was decided to investigate the interactions between atropine and fluphenazine and gamma-hydroxybutyrate and fluphenazine.

METHODS

Male wistar rats weighing 200 — 250 g were used in all the experiments.

Rigidity was induced by an intraperitoneal injection of 10 mg/kg of fluphenazine, which is chemically 2-trifluoromethyl-10 — {3′ — [4] — -(2 -hydroxyethyl)-1-priperazinyl] propyl} phenothiazine. Fluphenazine induced rigidity was palpated at dorsiflexion of the rat's foot and its onset, duration and intensity were observed.

Acetylcholine was extracted from individual rat brains by the method of Mac Intosh and Perry (1950). The extracts were assayed for acetylcholine on the guinea-pig ileum pretreated with mipafox.

Rigidity and brain acetylcholine were also estimated 45 minutes after intraperitoneal administration of either 10 mg/kg of atropine sulphate or 1,5 g/kg of gamma hydroxybutyrate into rats previously pretreated with fluphenazine for 15 minutes.

Rats injected with saline were used as controls.

RESULTS

Fluphenazine induced rigidity is manifested in rats by a decreased spontaneous motor activity, by a posture of general flexion and especially by an increased resistance against dorsiflexion of the feet and toes. This resistance was weak 5 minutes after the injection of fluphenazine. After that time it increased, reached a maximum 30 minutes after fluphenazine administration and then it started to decrease. 90 minutes after fluphenazine injection this resistance was still pronounced. The time course of fluphenazine induced change in rat brain acetylcholine is shown in Fig. 1. Fluphenazine induced an increase in rat brain acetylcholine concentration. The concentration was significantly higher than the control 15 minutes after the injection of fluphenazine ($p<0,05$). It continued to rise until 30 minutes after the injection when it reached a mean value of 5.35 μg/g ($p<0,001$), representing an increase over the control of about 165%. Sixty minutes after the injection of fluphenazine the brain acetylcholine concentration was still significantly higher ($p<0,001$) than that of saline controls.

Atropine and gamma hydroxybutyrate showed in our experiments the same effect on fluphenazine induced increase in the rat brain acetylcholine concentration, but had different effects on fluphenazine induced rigidity in rats. Atropine had no measurable effect on rigidity, and gamma-hydroxybutyrate abolished it.

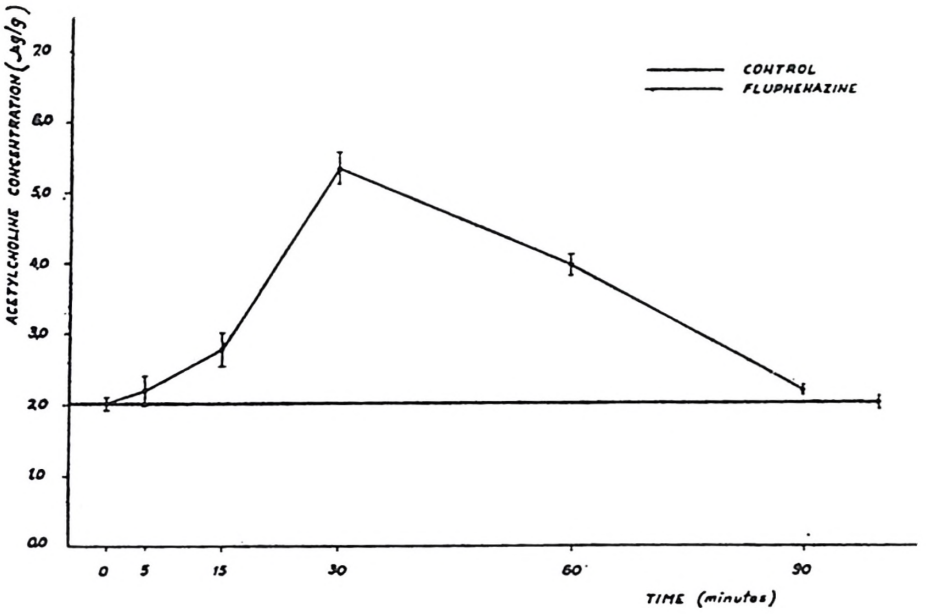

Fig. 1

The time course of fluphenazine induced change in rat brain acetylcholine concentration (µg/g whole brain ± standard error). Ordinate: acetylcholine concentration in µg/g. Abscissa: time in minutes. The horizontal line represents acetylcholine concentration of saline pretreated controls. Each point is the mean of five determinatoins.

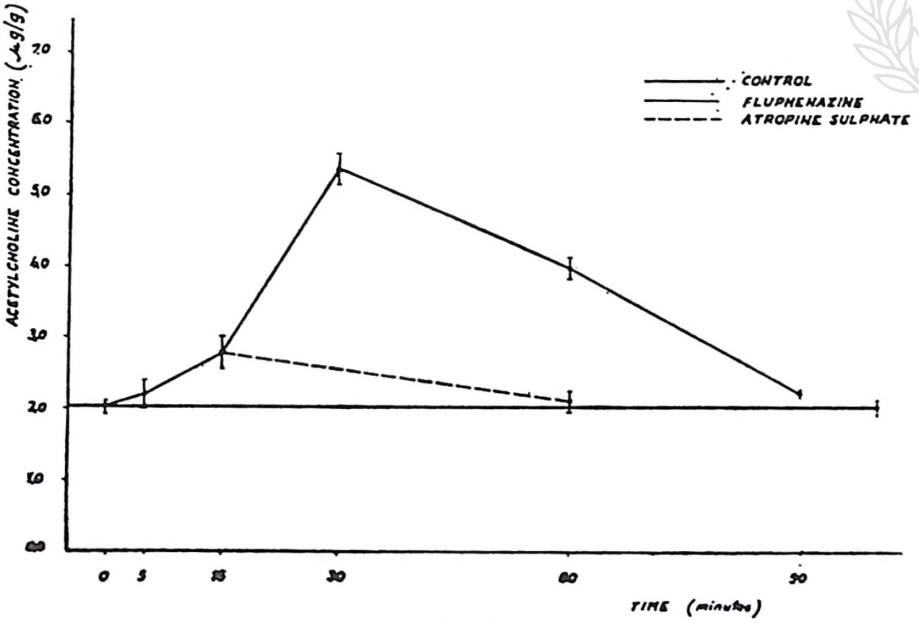

Fig. 2

Effect of atropine sulphate on fluphenazine induced increase in rat brain acetylcholine concentration (µg/g whole brain ± standard error).

The effect of atropine sulphate on fluphenazine induced increase in rat brain acetylcholine concentration is shown in Fig. 2. The effect of gamma-hydroxybutyrate on fluphenazine induced increase in rat brain acetylcholine concentration is shown in Fig. 3. As can be seen both drugs caused a significant decrease in fluphenazine induced rise in the rat brain acetylcholine. Mean values of 2.10 μg/g after atropine and 2.19 μg/g after gamma-hydroxybutyrate were not significantly diffe-

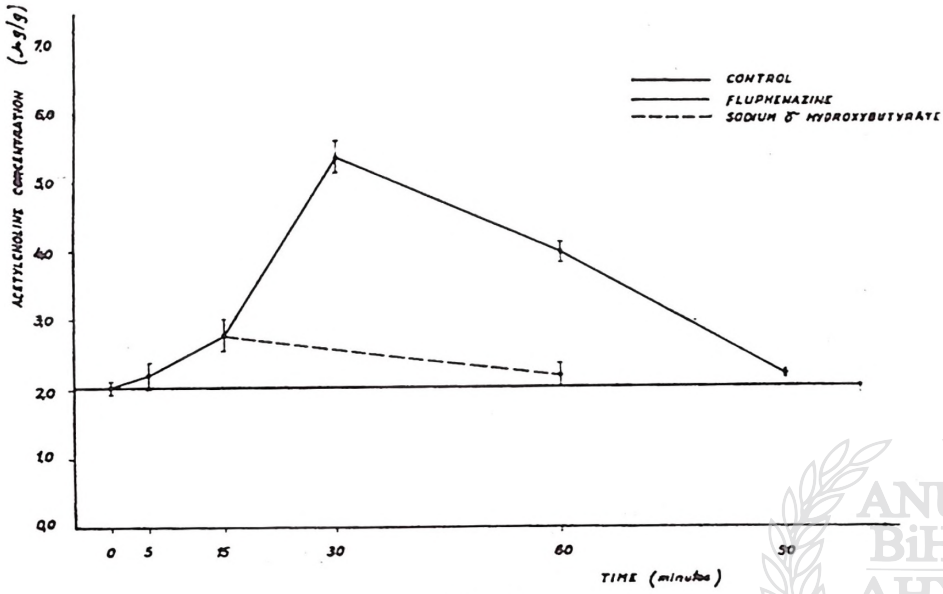

Fig. 3

Effect of gamma-hydroxybutyrate on fluphenazine induced increase in rat brain acetylcholine concentration (μg/g whole brain ± standard error).

rent from the saline control value, but representented a significant decrease of about 45% when compared with the corresponding mean value after fluphenazine alone.

DISCUSSION

It has been generally accepted (C a r l s s o n and L i n d q v i s t, 1963; R o o s and S t e g, 1964; H o r n y k i e w i c z, 1966 and 1967; J u r n a et al., 1969) that phenothiazine derivatives induce rigidity in experimental animals by an inhibition of monoaminergic transmission which is considered to be due to the blockade of catecholamines, especially at dopamine receptors in the brain. These substances thus prevent the endogenous dopamine from acting on its specific receptor and cause a functional dopamine dificiency at the synapses.

In our experiments, however, the phenothiazine derivative fluphenazine, which has been shown to be about 25 times more potent in

inducing rigidity than chlorpromazine which in this respect occupies an intermediate position among phenothiazine substances (Bohaček et al., 1963; Hornykiewicz, 1967) — produced a significant increase in rat brain acetylcholine concentration during the state of rigidity. The onset, intensity and duration of rigidity and the rise in brain acetylcholine concentration coincided with each other. This could mean that fluphenazine induced rigidity and the increase in brain acetylcholine were causally related and that fluphenazine might be able to produce rigidity by causing an imbalance between monoaminergic and cholinergic systems in the brain in two ways: by inhibiting monoaminergic transmission as well as by facilitating cholinergic transmission. At present little work has been reported concerning the effect of phenothiazines on acetylcholine concentration. There have been some reports that chlorpromazine reduced the activity of acetylcholine esterase (Kivalo et al., 1958). Bošković (1970) investigated the effect of fluphenazine on the acetylcholine esterase activity both in vitro and in vivo and he found that this substance caused a significant decrease in activity of acetylcholine esterase in vitro. He was unable, however, to detect any significant effect of fluphenazine on that enzyme in vivo by means of histochemical methods used. Thus the mechanism of fluphenazine induced rise in brain acetylcholine concentration in vivo is at present still uncertain and is yet to be explained. The results obtained with atropine and gamma-hydroxybutyrate seem to indicate that fluphenazine, might produce rigidity by causing an imbalance between monoaminergic and cholinergic functions in the brain in two ways: by inhibiting monoaminergic transmission as well as by facilitating cholinergic transmission.

Atropine has been reported to restore a drug induced shift of the monoaminergic-cholinergic equilibrium and abolish rigidity by depressing cholinergic functions (Arvidsson et al., 1966, Jurna et al. 1969). however, succeded to eliminate reserpine induced rigidity (which has been reported to be of the same type as phenothiazine rigidity) by pretreatment with atropine, but found it difficult to demonstrate an antagonising effect when atropine was applied after reserpine induced rigidity had fully developed. In our experiments atropine was administered into rats which already exibited fluphenazine induced rigidity and was not able to abolish it, but did produce a decrease in brain acetylcholine. The only possible explanation for these effects of atropine might be the already mentioned two ways fluphenazine induced shift in the monoaminergic-cholinergic eüuilibrium in the brain. Rigidity which persisted after atropine administration was probably due to the fluphenazine induced inhibition of monoaminergic transmission and atropine has not been shown to inhibit the monoaminergic transmission.

Results obtained with gamma-hydroxybutyrate give more support to the opinion that fluphenazine might produce rigidity by causing an imbalance between monominergic and cholinergic functions in the brain in two ways. Gamma-hydroxybutyrate has been reported to produce a selective, dose-dependent increase of brain dopamine in mammals (Gessa et al., 1966) and in our experiments it abolished induced rigidity and produced a decrease in brain acetylcholine, the mecha-

nism of which is still to be explained. Causing a selective increase of brain dopamine and a significant decrease in brain acetylcholine this drug restored the shift of monoaminergic-cholinergic equilibrium induced by fluphenazine — and abolished rigidity.

D. POTKONJAK

ODNOS IZMEĐU RIGIDITETA I PROMENA U KONCENTRACIJI UKUPNOG ACETILHOLINA IZ CELOG MOZGA ŠTAKORA IZAZVANIH POMOĆU FLUFENAZINA

KRATAK SADRŽAJ

Objavljeno je da supstance koje izazivaju pomeranje ravnoteže između monoaminergičnog i holinergičnog sistema u korist holinergičnih neurona (bilo facilitacijom holinergićnih ili inhibicijom monoaminergičnih neurona) u mozgu eksperimentalnih životinja (štakora) — mogu dovesti do pojave rigiditeta kod tih životinja.

Intraperitonealna primena flufenazina izazvala je rigiditet kod štakora. U isto vreme koncentracija ukupnog acetilholina iz celog mozga tih štakora bila je signifikantno povišena.

Atropin sulfat nije imao primetnog uticaja na rigiditet izazvan flufenazinom, ali je izazvao signifikantno sniženje koncentracije ukupnog acetilholina iz celog mozga štakora.

Gama-hidroksibutirat je potpuno uklonio rigiditet izazvan pomoću flufenazina i doveo je do signifikantnog sniženja koncentracije ukupnog acetilholina iz celog mozga tretiranih životinja.

Neki od ovih rezultata izgleda da upućuju na to da flufenazin može izazvati rigiditet prvenstveno pomoću facilitacije holinergičnog sistema, a u manjoj meri pomoću inhibicije monoaminergičnih neurona do koje dolazi zbog blokade dopaminskih receptora, što dovodi do funkcionalnog deficita dopamina u sinapsama mozga štakora.

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