enhance the activities of carbonic anhydrase and catalase in oats and beans[14]. However, the effect of pyridoxine on enzymes, including nitrate reductase (which occupies a key position in the nitrogen metabolism of plants), does not seem to have been studied so far. Our observation, that pyridoxine significantly increases leaf NRA levels in moong, assumes greater importance in the light of the positive correlation with seed yield noted in both experiments. As pyridoxine was also found to increase root growth and root nodule number (table 1), it may be argued that this effect facilitated a greater availability of nitrate[8], which is the inducer and stabilizer of nitrate reductase[15,16].

Comparison of the yield data of experiments 1 and 2 (table 2) clearly indicates the superiority of the soaking of seeds in pyridoxine solution, as it is more effective than spraying. Seed treatment also requires a smaller quantity of the vitamin for treating the same population of plants. Estimation of NRA is, moreover, a rapid and reliable technique for predicting crop productivity[12,13]. It is well established that yields may be augmented by appropriate remedial measures, including spraying of nutrients[17], if their deficiency is detected early enough; N deficiency, for example, may be indicated by NRA estimation. Seed treatment with very dilute pyridoxine solutions may, therefore, be exploited commercially as a simple, convenient and economical farm practice for ensuring higher productivity.

Table 2. Balance sheet for alternative methods of pyridoxine application in relation to seed yield of *Vigna radiata* var. K-851

Experimente No.	Remarks	Seed yield (q/ha)	Quantity of pyridoxine required (kg/ha)
1	I) Optimum seed treatment (0.3% pyridoxine)	13.82	0.09
	II) Water-soaked control	8.92	–
	III) Increase over control I–II	4.90	–
2	IV) Optimum spray treatment (0.1% pyridoxine)	12.69	2.00
	V) Water-sprayed control	9.49	–
	VI) Increase over control IV–V	3.20	–

Economics of seed treatment versus spray treatment

a)	Increase in seed yield III–VI	1.70 q/ha
b)	Price of additional yield at $40/ha	$68.00
c)	Saving on pyridoxine IV–I	1.91 kg/ha
d)	Price of pyridoxine saved at $80/kg	$152.80
e)	Net profit b + d	$220.80/ha

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Comparison of the effects of different isomers of bicuculline infused in the preoptic area on male rat sexual behavior

A. Fernández-Guasti, K. Larsson[1] and C. Beyer[2]

Department of Psychology, University of Göteborg, Box 14158, S-400 20 Göteborg (Sweden), and Centro de Investigación en Reproducción Animal, CINVESTAV-UAT, Ap. Postal 62, Tlaxcala 90-000 (México), 25 October 1984

Summary. Intracerebral infusion of (+) bicuculline methiodide, but not of its (−) isomer, in the preoptic area, stimulated masculine sexual behavior in rat as evidenced by a decrease in the number of intromissions preceding ejaculation and a shortening of the ejaculation latency and postejaculatory interval. Data suggest a role of the GABAergic system in mediating masculine sexual behavior.

Key words. (+) and (−) bicuculline methiodide; masculine sexual behavior; preoptic-anterior hypothalamic area; rat.

The masculine sexual behavior in the rat is an innate, stereotyped behavior pattern: the male repeatedly mounts and penetrates the receptive female until ejaculation is achieved; he then becomes unresponsive to sexual stimulation for a period which becomes progressively longer with each successive ejaculation[3]. Only little is known about the neuroendocrine processes underlying this behavior. The medial preoptic-anterior hypothalamic continuum (MPOA) is of critical importance since lesions in this area abolish sexual behavior[4] whereas electrical stimulation of it facilitates the elicitation of copulation[5,6]. Efforts have been made to study the neurotransmitters involved in determining this behavior, the interest mainly focussed on the role of brain monoamines[7]. It is generally agreed that depression of central 5-hydroxytryptamine (5-HT) activity results in a facilitation of the masculine sexual behavior in rats while central 5-HT stimulation has an opposite effect[8]. The importance of dopamine and noradrenaline for sexual activity is less well established[7]. Recent evidence also indicates a role of the cholinergic system in male rat sexual behavior[9].

Little attention has been given to a possible role of amino acids in the mediation of sexual behavior, although in quantitative terms, amino acids now appear to be the major neurotransmitters in the mammalian central nervous system[10]. As a inhibitory neurotransmitter, gamma aminobutyric acid (GABA) ap-

pears to be of particular importance in the brain[11]. Bicuculline methiodide has been effectively used as a GABA antagonist[12]. Two isomeric forms of this compound occur: a (+) form having a GABAergic antagonistic effect, and a (−) form which does not antagonize GABAergic transmission[13,14]. We therefore decided to infuse each of these compounds into the MPOA and to analyze their effects on masculine sexual behavior.

Materials and methods. Male Wistar rats (300–350 g b.wt) were selected for their ability to execute the complete male copulatory behavior pattern. Selected males were bilaterally implanted under pentobarbital anesthesia (40 mg/kg), with two guiding cannulae aimed at the brain surface corresponding to the MPOA. Implanted animals were divided into three groups and received one of the following treatments: 30 ng/cannula (+) bicuculline methiodide (Pierce), 30 ng/cannula (−) bicuculline methiodide (Sigma), or 0.5 µl saline. (+) and (−) bicuculline methiodide were dissolved in saline and injected in 0.5 µl. Animals were tested for male sexual behavior immediately after drug or saline infusion. Estrogen-progesterone treated females were used as stimulus. The male was presented with the receptive female in a circular (59 cm diameter) plexiglass arena. Standard parameters of male copulatory behavior were recorded through three ejaculatory series as follows: intromission frequency (number of intromissions preceding ejaculation); ejaculation latency (time between the first intromission and ejaculation); and postejaculatory interval (time from ejaculation to the first intromission of the next series). By series of copulation is meant the number of mounts and intromissions preceding ejaculation. After completion of testing, the animals were injected with fast green through the guiding cannulae. Brains were removed and the injection site localized. Data from animals with marks outside the MPOA were not included in the experimental analysis. Data were statistically analyzed using Mann Whitney U-test[15].

Results. Figure 1 shows the placement of the injection cannulae in the three groups of animals used in this experiment. As indicated in the figure, all cannulae were localized within the preoptic area. Figure 2 shows the results of this study. Intracerebral infusion of the (−) form of bicuculline methiodide did not produce any deviations in the mating pattern compared to that displayed by saline-treated controls. However, in its (+) form, bicuculline methiodide drastically changed the mating pattern. The number of intromissions preceding ejaculation was significantly reduced in the first series of copulations, and the ejaculation latency was significantly shortened in the first and second series. Even more remarkable changes were observed in the postejaculatory intervals. Among the saline-treated controls, none of the animals resumed mating earlier than a minimum of 4, 5 and 6 min after the 1st, 2nd and 3rd ejaculation respectively while in the (+) bicuculline methiodide treated animals, all but one male started to copulate within 1 min after the first ejaculation. The refractory periods following the second and third ejaculations were also reduced below normal levels. After the second and third ejaculations, the median lengths of the refractory periods were 3.1 and 3.8 min compared to 6.2 and 8.1 min in the controls. In one particular test, 8 ejaculations were recorded in 10 min. None of the refractory periods shown by this rat were longer than 1.05 min.

Discussion. Results clearly show that infusion in the MPOA, of (+) bicuculline methiodide stimulates masculine sexual behavior in the rat, while (−) bicuculline methiodide has no such effect. Since (+) bicuculline methiodide produces neuronal excitation[13], the stimulatory effect of this compound on the sexual behavior might be an unspecific action caused by increased firing of the

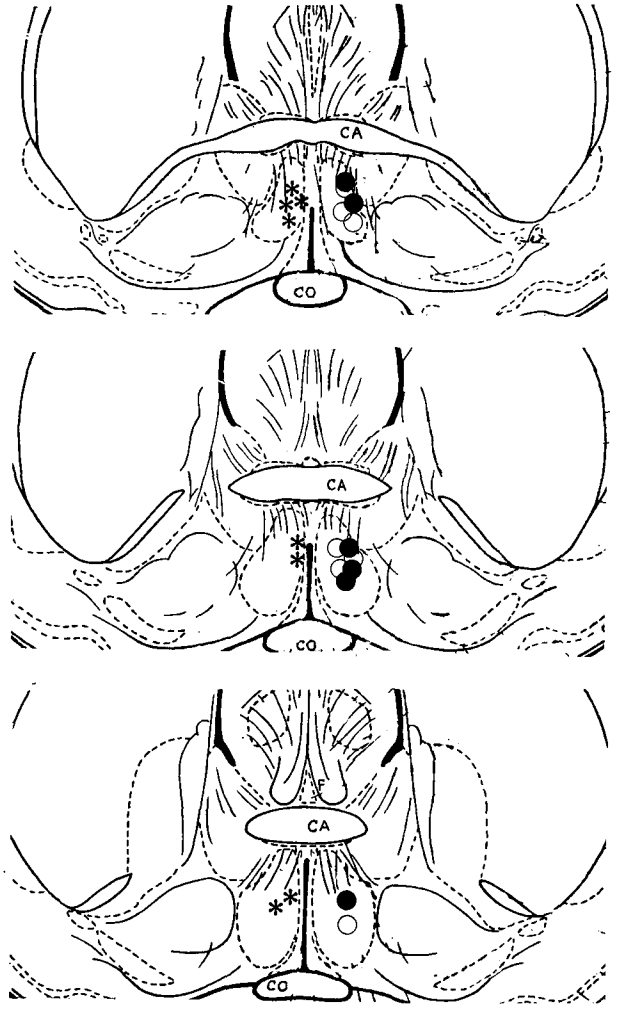

Figure 1. Schematic representation of injection sites in the medial preoptic area (MPOA). ○ (+) bicuculline methiodide; ● (−) bicuculline methiodide; * saline. CA: Commissura anterior, CO: Chiasma opticum (after König and Klippel, 1967)[21].

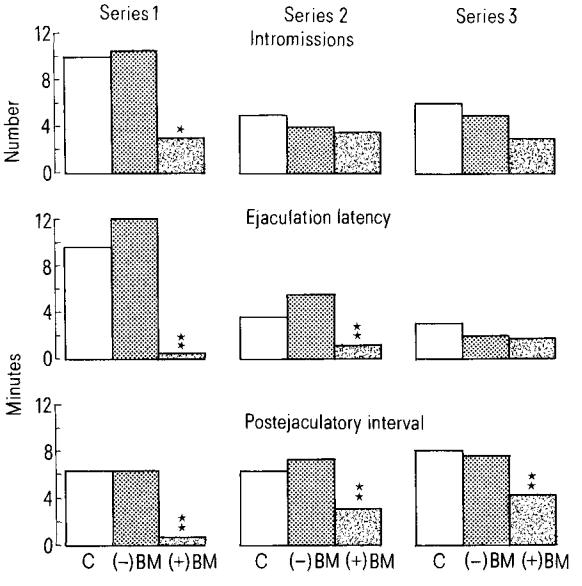

Figure 2. Effects of intracerebral infusion of 0.5 µl saline (C, n = 9); 30 ng/cannula (−) bicuculline methiodide ((−) BM, n = 6); 30 ng/cannula (+) bicuculline methiodide ((+) BM, n = 7) on masculine rat sexual behavior. Figure shows median values. Mann Whitney U-test, * $p < 0.05$; ** $p < 0.02$.

neurones in this brain area. However, (−) bicuculline methiodide stimulates neuronal firing without antagonizing the action of GABA in several neural systems[13,14]. Therefore, the action of (+) bicuculline methiodide on masculine sexual behavior seems to be specifically associated with its property of antagonizing a GABAergic inhibitory input on the neural substrates of this behavior.

The antagonizing effects of (+) bicuculline methiodide appear to be GABA specific; this compound, for example, does not interfere with the action of noradrenaline and 5-HT on neuronal activity[16]. However, since monoamines have been implicated as neurotransmitters in mediating masculine sexual behavior[7,8], and GABAergic neurons are known to interact with monoaminergic neurons[17], the possibility that (+) bicuculline methiodide stimulates sexual behavior by interfering with the interaction between GABA and brain monoamines cannot be excluded from the present findings.

Comparing the present findings with those following treatment with drugs affecting the central monoamine transmission[7,18-20], (+) bicuculline methiodide influences both the copulatory series and the postejaculatory intervals. Recently, McIntosh and Barfield[18] reported a shortening of the postejaculatory interval following either electrolytic or chemical lesions selectively affecting the serotoninergic system and suggested the involvement of this system in the control of the length of the postejaculatory interval. However, the effect of (+) bicuculline methiodide on the postejaculatory interval is far more pronounced than that observed after interference with the central monoaminergic system.

1 Reprint requests to K.L., Department of Psychology, Unit of Psychobiology, University of Göteborg, Box 14158, S-400 20 Göteborg, Sweden.
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The effect of sulfhydryl compounds on the catalytic activity of Cu, Zn-superoxide dismutase purified from rat liver[1]

T. Hoshino, Y. Ohta and I. Ishiguro[2]

Department of Biochemistry, Fujita-Gakuen Health University School of Medicine, Toyoake, Aichi 470-11 (Japan), 29 August 1984

Summary. Sulfhydryl compounds such as reduced glutathione, cysteine and 2-mercaptopropionylglycine, a hepato-protective agent, activated Cu, Zn-superoxide dismutase purified from rat liver at low concentrations (below 10 μM). Furthermore we found evidence indicating that this activation is achieved by reducing Cu^{2+} present in the catalytic site of the dismutase, and thereby promoting the dismutation of superoxide anions.
Key words. Reduced glutathione; cysteine; 2-mercaptopropionylglycine; Cu, Zn-superoxide dismutase (rat liver).

Superoxide dismutase (SOD) (EC 1.15.1.1) is a metalloprotein and catalyzes the dismutation of superoxide anions (O_2^-) to hydrogen peroxide (H_2O_2) and molecular oxygen[3]. It has been shown that in mammalian tissue cells, SOD is distributed in the mitochondria and cytosol and exists in two forms, i.e., the Cu- and Zn-containing enzyme in both the intermembrane space of mitochondria and the cytosol and the Mn-containing enzyme in the matrix space of mitochondria[4]. It has been demonstrated that Cu, Zn- and Mn-SODs operate a redox cycle in which the active site metal, the Cu in the Cu, Zn-enzyme, is alternately reduced and reoxidized by O_2^-[5,6]. SOD is also widely recognized to play an important role in protecting the cell against oxygen toxicity[7].

Besides SOD, reduced glutathione (GSH) and cysteine, which are thiols present in nature, have been demonstrated to react with O_2^- nonenzymatically in vitro, and it has been thus suggested that these SH-compounds might play a major role in controlling O_2^- concentrations together with SOD in vivo[8,9]. 2-Mercaptopropionylglycine (MPG), which is a synthetic amino acid containing one SH-group that is used clinically as a hepato-protective agent, is reported to have chemical, biochemical and pharmacological properties similar to GSH[10]. This synthetic SH-compound has also been found to function as a scavenger of O_2^- in vitro[11,12]. However, although it is well known that SH-compounds form complexes with metal ions such as Cu^{2+} and Zn^{2+} and that they transfer one electron to metal ions such as Cu^{2+}[13,14], there has been no available information on the direct interaction between these SH-compounds and Cu, Zn- and Mn-SODs.

In the present study, we examined how SH-compounds such as GSH, cysteine and MPG affect the catalytic activity of Cu, Zn-SOD purified from rat liver. These SH-compounds were found to activate the dismutase at low concentrations. The mechanism for this activation was further investigated.