Effects of intraventricular carbachol and eserine on drinking[1]

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Rats implanted with chronic cannulae in the lateral cerebral ventricle were treated with carbachol and eserine. Five doses of each drug were given to determine their effects on water intake of the Ss. All doses of carbachol and four lower doses of eserine failed to affect drinking. The findings suggest that drinking after application of carbachol to CNS structures, implicated in the thirst circuit, is not due to ventricular modification.

It has been demonstrated that direct application of the cholinomimetic substance, carbachol, to a number of subcortico-diencephalic brain structures results in excessive drinking in the stimulated rats (Fisher & Coury, 1960; Coury, 1967). Carbachol-induced drinking occurs from anatomically diffused brain regions which are located near the brain midline along the horizontal plane. In addition, brain regions implicated in the central thirst circuit are generally in close proximity to the cerebral ventricular system (Feider, 1965). These facts have apparently led to the formulation of a ventricular modification hypothesis to account for carbachol-induced drinking (Routtenberg, 1967). The hypothesis advocated that perhaps the central drinking circuit, as determined by carbachol application, is not as diffused as it appears to be and ventricular participation can account for the phenomenon. This study was carried out to evaluate the dose-response effects of a cholinomimetic (carbachol) and an anticholinesterase (eserine) on drinking, when the compounds were delivered directly into the lateral cerebral ventricle of rats.

Method. The Ss were 30 male albino rats of the Sprague-Dawley strain, between 120-150 days old at the beginning of the experiment. All Ss were stereotaxically implanted with unilateral chronic cannulae (Khavari, Feider, Warburton, & Martin, 1967) in the lateral cerebral ventricle. Surgical procedures were essentially the same as reported previously (Khavari & Maickel, 1967). Coordinates employed (from bregma) were: H = 5.5 mm, L = 2.5 mm, V = 0.9 mm posterior. Rats were allowed to recover from surgery for approximately 10 days prior to the start of the experiment. At the end of the study, brains were removed and location of cannulae tips were verified to be in the lateral ventricle.

The apparatus consisted of stainless steel cages of 8 x 8 x 11 in., i.e., the Ss' home cages. All Ss were maintained on a 23-h water deprivation schedule. Dry laboratory rat food was available at all times except during the 1-h test session. In each daily test session the S had access to tap water, for 1 h, in its home cage. After approximately 10 days body weight and water intake of the Ss became stabilized, and they were randomly assigned to three equal groups. Five doses were tested with each drug: carbachol (.25, .5, 1, 2, and 4 μg/rat); and eserine (.5, 1, 2, 3, and 4 μg/rat). All drug and saline injections were administered intraventricularly immediately prior to the 1-h test sessions. Distilled water was used as the vehicle for all drug injections. The volume injected, for both saline and drug, was always 10 μl/rat. Each rat received the following sequential treatments: The first day constituted the No Injection session in which the rat's water intake was measured at the end of the 1-h session; the second day was the Predrug session in which the S received an intraventricular injection of physiological saline prior to the test; the third day was the Drug day in which the rat was given its assigned dose of the drug; in the fourth day, Postdrug, the S was injected with saline before the test; and finally, for a 3-day period the Ss were treated the same way as the No Injection day, i.e., in any given week there were four no injection, two saline, and one drug days.

Table 1 presents: doses of carbachol and eserine used; mean values of intake, in ml, and standard errors for Predrug, Drug, and Postdrug session; and statistical significance of the difference, between the three conditions, as determined by correlated t test.

Results and Discussion. The results are summarized in Table 1. None of the five doses of carbachol affected water intake of the Ss significantly. The data failed to support Routtenberg's notion (1967) that carbachol-produced polydipsia in the rat may be due to ventricular modification. Grossman (1962), Feider (1965) and others have reported that carbachol induces excessive drinking, in sated as well as water-deprived rats, when the cholinomimetic substance is applied to structures implicated in the central drinking circuit. Clearly, the present data rule out the ventricular system as mediator of carbachol effect, a finding independently reported recently (Myers & Cicero, 1968).

Table 1
Mean Water Intake in ml for Predrug, Drug, and Postdrug Days (Standard errors are given in parenthesis)

Drug and Dose (μg/rat)	Intake and (S.E.M.) Predrug	Drug	Postdrug
CARBACHOL			
0.25	24.2 (1.27)	27.2 (3.70)	23.7 (0.78)
0.50	24.8 (0.64)	22.7 (2.63)	23.4 (1.00)
1.00	23.4 (1.43)	22.6 (2.08)	24.6 (1.97)
2.00	24.4 (0.47)	20.8 (2.41)	26.5 (1.30)
4.00	21.8 (0.85)	19.5 (2.25)	24.5 (1.37)
ESERINE			
0.50	23.7 (1.71)	25.1 (1.72)	25.1 (1.95)
1.00	24.2 (0.81)	22.4 (1.05)	23.7 (1.2)
2.00	23.1 (0.93)	20.6 (1.38)	24.3 (0.85)
3.00	23.1 (1.41)	24.6 (0.99)	24.1 (1.07)
4.00	24.6 (0.63)	20.7 (0.89)*	21.8 (1.04)

**Significantly different from predrug, $p < .05$.*

The first four doses of intraventricular eserine also failed to affect the water intake of the Ss significantly (Table 1). Only the high dose of 4 μg/rat produced statistically significant change, i.e., it reduced the Ss' intake. Reduction of water intake under the last dose of eserine may be due to factors other than direct anticholinesterase properties of the drug as related to the thirst circuit. Miller (1965) has reported that injection of eserine into the preoptic region of slightly water-deprived rats resulted in an increase of more than ten fold in their drinking. This is indeed what one would expect if the notion that drinking is mediated by a central cholinergic circuit is to be substantiated, i.e., while cholinomimetic and anticholinesterase compounds increase drinking the anticholinergic drugs should decrease it when applied to any point in the functional circuit. However, recent data from this laboratory indicate that the neurochemical substrate of thirst is much more complex than was originally conceived, e.g., intraventricular atropine produced a highly reliable and dose-dependent reduction in drinking of water-deprived rats, while carbachol and eserine generally failed to influence water intake. Therefore, on the basis of the present data the hypothesis that ventricular modification may be responsible for carbachol-induced excessive drinking (Routtenberg, 1967) does not hold, a fact independently verified recently (Myers & Cicero, 1968). In addition, a general and oversimplified postulation of a central cholinergic thirst circuit seems to require complete reevaluation.

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NOTE

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Reply to Bignami

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There appears to be no dispute between Bignami and me concerning what I believe to be the major points about muscarinic anticholinergic agents and passive avoidance in the rat. A particular demonstration of the absence of an effect on passive avoidance by such an agent (Meyers & Koenig, 1967) occurring within a context of studies using different behavioral situations and reporting a disruptive effect for these agents (Bignami, 1967; Bovet, Robustelli, & Bignami, 1965; Meyers, 1965; etc.) strongly suggests that anticholinergic drugs produce no general inability to avoid passively. Rather, it indicates that only in certain situations do anticholinergic drugs impair passive avoidance. The conditions under which a deficit will or will not be observed remains to be elaborated and, of course, the efforts to do this, which Bignami remarks are now underway in his laboratory, are to be welcomed.

It is obvious, as Bignami indicates, that the presence of an external signal for the shock contingency does not guarantee the lack of an effect by anticholinergic agents on passive avoidance behavior. Thus, the discrepant findings observed in various studies which used externally-cued passive avoidance tasks must have resulted from one or more of the many procedural differences which existed among these studies (Bignami, 1967; Bovet et al, 1965; Meyers & Koenig, 1967).

Finally, Bignami and I are in complete agreement that no single mechanism is currently available to explain the literature for anticholingeric drugs and that recourse to analogy with lesion effects might be fruitful.

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