Physiology and Behavior. Vol. 2, pp. 367–371. Pergamon Press Ltd., 1967 Printed in Great Britain

Changes in Body Temperature and Water Intake following Intracerebral Implantation of Carbachol in Rats

S. G. T. HULST AND D. DE WIED

Department of Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, Utrecht, The Netherlands

(Received 25 March 1967)

HULST, S. G. T. AND D. DE WIED. *Changes in body temperature and water intake following intracerebral implantation of carbachol in rats.* PHYSIOL. BEHAV. **2** (4) 367–371, 1967.—Intracerebral carbachol produced a fall in core temperature in the rat when implanted in the area preoptica, the nucleus lateralis septi and the area between the thalamic nuclei and the nucleus ruber. Cholinergic stimulation of the anterohypothalamic region did not affect body temperature, while stimulation of the nucleus ventralis thalami induced hyperthermia. Water consumption increased upon intracerebral application of carbachol to the area preoptica, the nucleus lateralis septi, the anterohypothalamic region and the area between the thalamic nuclei and the nucleus ruber. No effect was found following implantation of carbachol in the nucleus ventralis thalami. The results suggest that drinking and hypothermia follow roughly parallel pathways in the limbic system and the diencephalon, although the fiber systems for these two phenomena need not be necessarily the same.

Water intake Body temperature Carbachol Intracerebral implantation

IN THE water satiated rat intracerebrally implanted carbachol elicits vigorous drinking [4, 5, 8, 12]. Grossman's demonstration that cholinergic stimulation of the perifornical region in the rat led to a marked increase in water ingestion, was further investigated by Fisher and Coury [2] who found that cholinergic stimulation within the limbic system, the hypothalamus and the midbrain also induced drinking. These authors postulated that the Papez circuit is intimately involved in the mediation of drinking. Recently, hypothermia in the rat has been found following cholinergic stimulation of the lateral hypothalamus [10] and of the preoptic region [8], but other structures in the central nervous system have not been implicated in this respect. The present experiments were conducted to determine the effect of carbachol on core temperature and on water ingestion after its administration into various subcortical structures.

MATERIALS AND METHODS

Male white rats of an inbred Wistar strain weighing 180–210 g were used.

To guide implantation of carbachol into various brain structures a stainless steel plate with 12 holes was used (Fig. 1). Tubes with a dia. of 0.80 mm of the same material of the plate protruding 2.50 mm from the lower side of the plate were attached to the holes in the plate. In order to fixate the plates to the skulls, rats were placed in a stereotaxic apparatus under ether anesthesia. After cutting the skin and cleaning the bone, holes—corresponding to the holes of the plate—were drilled in the skull starting just rostral of the sutura coronaria. After cleaning and drying the surface of the skull and following application of sulfonamide (Orgasepton) to avoid infection, the tubes of the plate were carefully inserted into the holes until the plate touched the skull. The plate was subsequently fixed to the skull with dental cement. Through the tubes, needles containing crystalline carbachol at the tip could be directed into the brain.

Rats were kept in individual cages at an environmental temperature of 24 ± 1°C. Food and water were available *ad libitum*. Water bottles were exchanged for a calibrated pipet. Animals were allowed to recover from the operation and to get used to the environment, and to the drinking from the pipet for one week. Rats in which the plate was not well fixed, or animals with aggressive behavior, severe loss of body weight, or abnormal body temperature were discarded from further participation in the experiments.

On the day of the experiment, an 18 gauge needle, adapted for this purpose containing a small amount of crystalline carbachol (approximately 5 μg) at the tip, was placed into the brain of the conscious rat (Fig. 2). The needles were cut at different lengths so that the carbachol could be placed at different depths into the brain. Sham-implantation was performed with similar needles at similar places without carbachol at the tip of the needle. The experiment lasted 4½ hr. Every 30 min following implantation of the carbachol a probe of a galvano-electric thermometer (Ellab Instruments

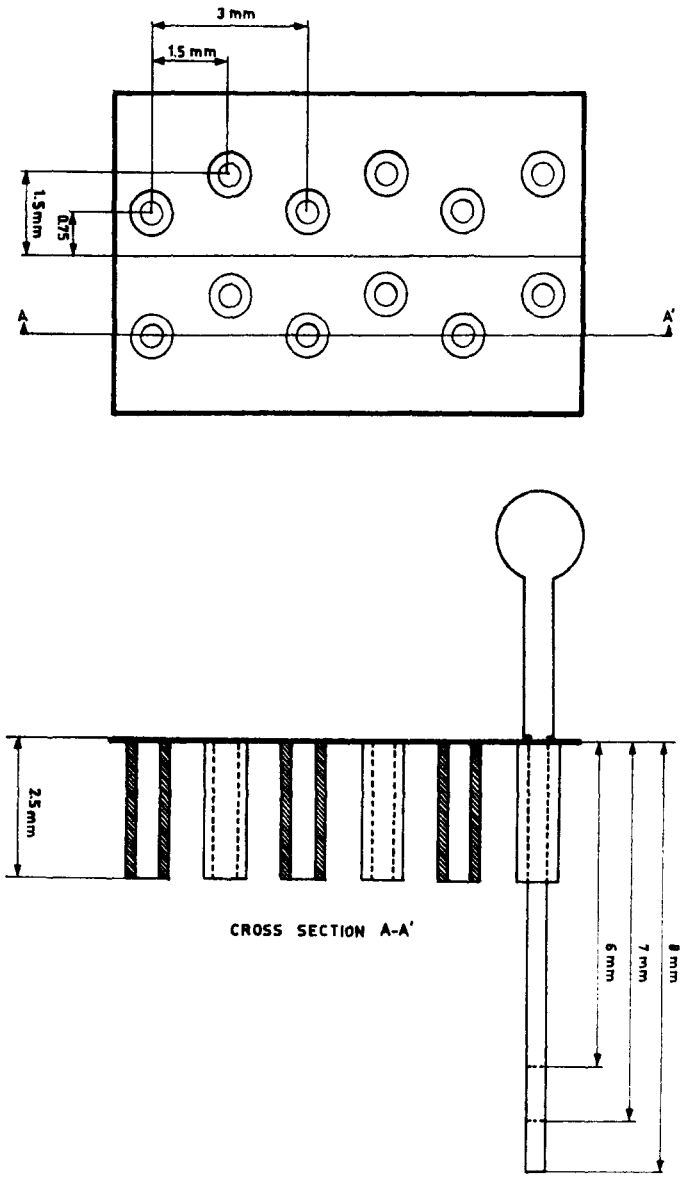

FIG. 1. Schematic representation of stainless steel plate with the 12 holes used to implant carbachol into various brain structures. Upper part shows location of the 12 holes. Lower part is a cross section, showing the tubes protruding from the lower side of the plate. Needles of different length were used. Drawing shows needle in place.

type TE 3) was inserted 5 cm deep into the rectum of the rat for approximately 2 min. Temperature was read and at the same time water intake was measured.

Rats were used only once a week and, depending on the condition of the animal, experiments were continued for approximately 7 weeks. Thereafter, animals were sacrificed. The places which had been reached by the needle were stained with Evans Blue. Brains were sectioned to examine the localization of these places using De Groot's atlas for the ratbrain as a reference [3].

RESULTS

Table 1 summarizes effects on temperature and water intake. The table contains results only of areas in which according to localization studies, three or more carbachol implants were found close to each other. Following carbachol implantation a marked and rapid decrease in core temperature occurred in several subcortical areas. A consistent fall in temperature was found after cholinergic stimulation of the area preoptica, the nucleus lateralis septi and the area between the thalamic nuclei and the nucleus ruber. Core temperature failed to respond to carbachol if the drug was implanted into the anterohypothalamic region. A significant increase in body temperature was found if carbachol was placed in the nucleus ventralis thalami. In none of the instances sham-implantation altered core temperature for more than 0.5°C. Figure 3 shows the effect on core temperature of carbachol implantation at the injection sites in the brain.

FIG. 2. Photograph of conscious rat with stainless steel plate *in situ* and needle for implantation inserted into the brain via one of the holes in the plate.

(*facing page* 368)

TABLE 1

EFFECT OF INTRACEREBRAL CARBACHOL ON CORE TEMPERATURE AND ON WATER INTAKE

Brain structure	Maximal change in temperature (°C per 4½ hr)	Mean	Maximal Water intake (ml per 4½ hr)	Mean
N. lat. septi	−1.0; −1.3; −3.5; +0.7; −2.0; −2.5; −2.0; +0.4	−1.4	11.2; 15.7; 7.4; 15.8; 15.0; 6.5; 14.6; 19.0	13.2
Area preoptica	−2.6; −2.0; −1.5	−2.0	8.2; 12.0; 15.0	11.7
Area anterohypothalami	−0.8; −0.3; +0.8	−0.1	19.2; 10.0; 10.0	13.1
Area between n. ventralis thalami and n. ruber	−1.8; −1.7; −1.7	−1.73	11.0; 12.2; 8.9	9.9
N. ventralis thalami	+2.5; +2.0; −0.7; +0.8; +0.5; +1.8; +1.0	+1.1	1.2; 1.0; 4.6; 3.0; 1.8; 6.3; 0.8	2.8

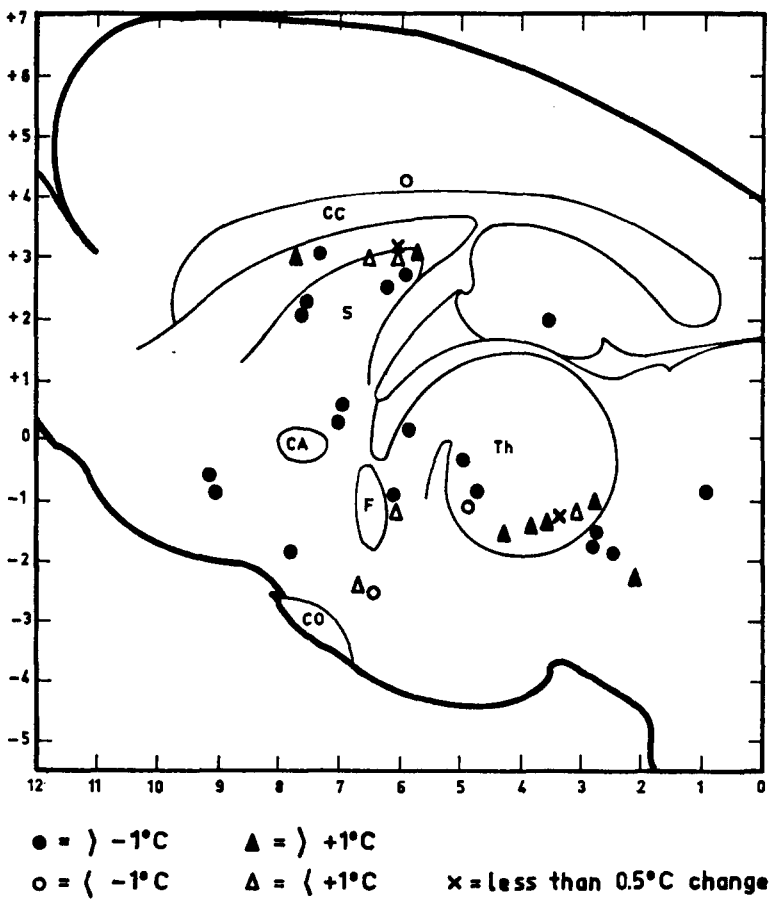

FIG. 3. Effect on core temperature of carbachol implantation at various injection sites in the brain. CC = Corpus callosum; S = Septum; Th = Thalamus; CA = Commissura anterior; F = Fornix; CO = Chiasma opticum.

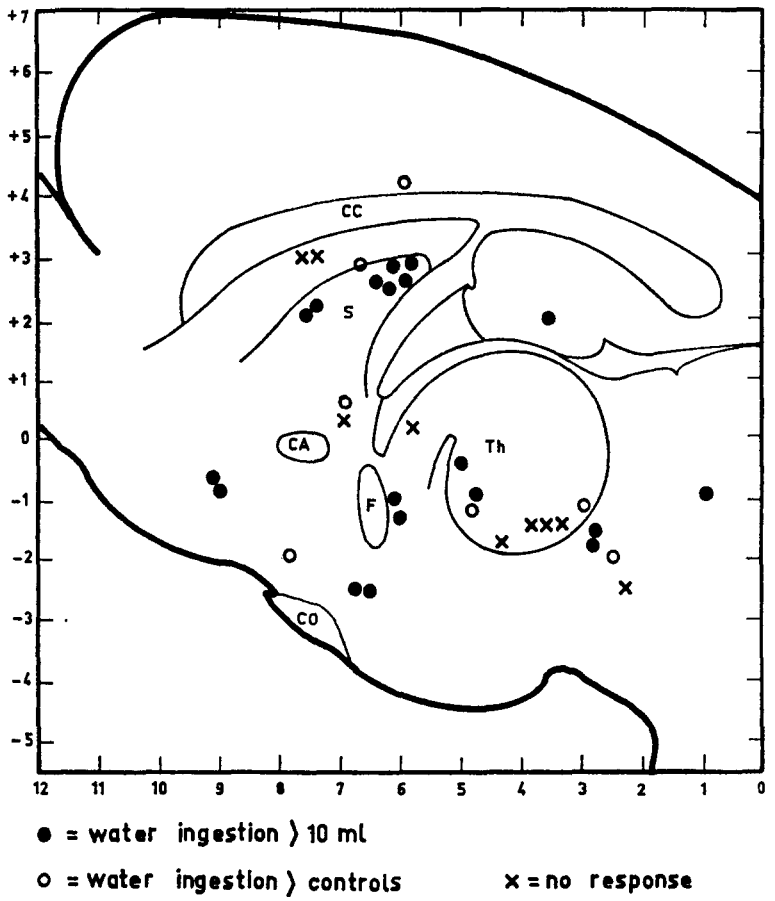

FIG. 4. Effect on water intake of carbachol implantation at various injection sites in the brain. S = Septum; CC = Corpus callosum; F = Fornix; CA = Commissura anterior; CO = Chiasma opticum; Th = Thalamus.

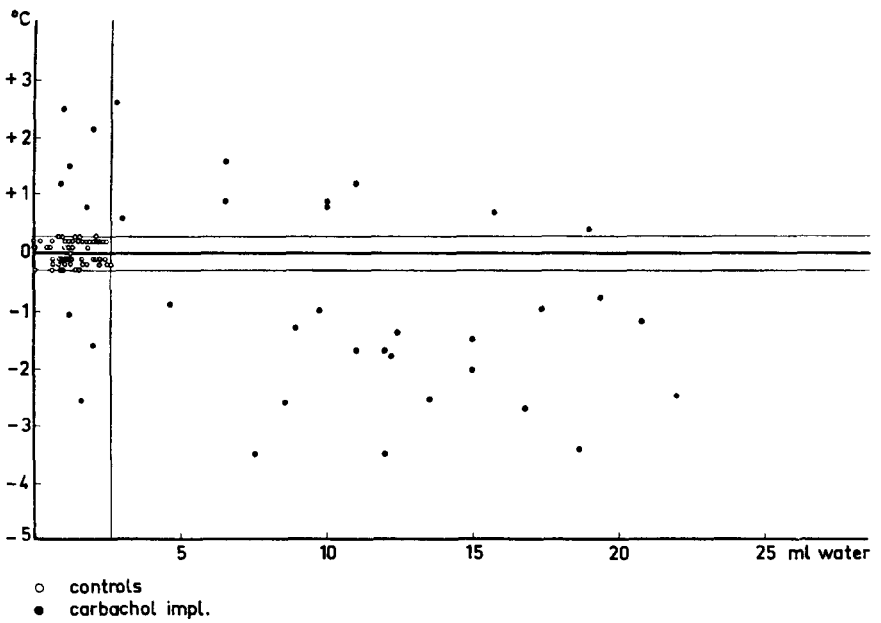

FIG. 5. Diagram showing relation between water intake and change in body temperature following carbachol implantation into the various subcortical areas. Results are expressed as maximal effects obtained during the period of 4½ hr of observation.

Cholinergic stimulation of several of the subcortical areas in the brain evoked water consumption. As seen from Table 1, the area preoptica, the nucleus lateralis septi, the anterohypothalamic area, and the area between the thalamic nuclei and the nucleus ruber all were sensitive to carbachol in this respect. However, application of carbachol to the nucleus ventralis thalami did not affect water consumption. Figure 4 shows the effect on water consumption of carbachol implantation at the injection sites in the brain.

The results indicate that cholinergic stimulation of a number of subcortical structures induces both a fall in core temperature and an increase in the rate of water intake. However, a fall in core temperature did not always parallel an increase in water consumption. Thus, the stimulation of the anterohypothalamic area which failed to affect body temperature, elicited an increase in water ingestion. This is clearly demonstrated in the diagram of Fig. 5 in which water intake is plotted against the change in core temperature. In 18 cases a fall in core temperature parallelled an increase in water intake. However, in 4 cases a decrease in temperature between 0.75 and 2.60°C was not accompanied by drinking. A rise in core temperature was found in 13 cases. In 5 of these water consumption was markedly enhanced.

DISCUSSION

The data accumulated thus far confirm results of several authors who demonstrated that cholinergic stimulation of various subcortical structures elicits drinking in the water satiated rat [2, 4, 5, 9]. Fisher and Coury [2] proposed a correlation between areas from which positive drinking responses were elicited and parts of the emotion-motivation limbic circuit postulated by Papez [11]. The present experiments are in accordance with the observation that cholinergic stimulation of several structures of the Papez circuit elicits drinking. Cholinergic stimulation of these structures also induced a change in core temperature. Apparently, structures belonging to the emotion-motivation limbic circuit are involved not only in the regulation of drinking but also in the control of core temperature. The number of observations however do not permit to conclude that hypothermia, like drinking, is mediated by fiber systems which are connected with the Papez circuit for emotion and motivation. A more complete mapping of positive regions is necessary.

Andersson and Larsson [1] have reported that local warming of the preoptic area induces the goat to drink large quantities of water. The present experiments indicate that fiber systems for drinking are partly different from those of hypothermia since cholinergic stimulation of the anterohypothalamic region induced drinking but not hypothermia. In addition, it was found that application of carbachol to the nucleus ventralis thalami induced hyperthermia without affecting water consumption. It seems that facilitatory and inhibitory connections both of cholinergic character may be involved in temperature regulation. In this respect it is worth noting that Grossman and Grossman [6] have shown that cholinergic stimulation of amygdalar areas can inhibit and facilitate drinking in water deprived animals.

Hernández-Peón [7] working with cats found a correlation between cholinergic stimulation and sleep. The sleep circuit in the cat seems to follow many of the same pathways as drinking and hypothermia in the rat. In fact, in most of the rats in the present experiments carbachol implantations led to a sleeplike state some hours after the intracerebral application of the drug. Accordingly, many of the areas implicated in studies on food and water intake, on sleep and in the present study also on temperature are in the same region of the CNS. Evidence is accumulating that circuits mediating these phenomena follow roughly parallel courses through the limbic system and the diencephalon, although the fiber systems for these phenomena need not be necessarily the same.

REFERENCES

1. Andersson, B. and B. Larsson. Influence of local temperature changes in the preoptic area and rostral hypothalamus on the regulation of food and water intake. *Acta physiol. scand.* **52:** 75–89, 1961.
2. Fisher, A. E. and J. N. Coury. Chemical tracing of neural pathways mediating the thirst drive. In: *Thirst*, edited by M. J. Wayner. Oxford: Pergamon Press, 1964, pp. 515–528.
3. De Groot, J. The rat forebrain in stereotaxic coordinates. In: *Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen LII no.* 4. Amsterdam: North-Holland, 1959.
4. Grossman, S. P. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. *Science* **132:** 301–302, 1960.
5. Grossman, S. P. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. *Am. J. Physiol.* **202:** 872–882, 1962.
6. Grossman, S. P. and L. Grossman. Food and water intake following lesions or electrical stimulation of the amygdala. *Am. J. Physiol.* **205:** 761–765, 1963.
7. Hernández-Peón, R., G. Chávez-Ibarra, P. J. Morgane and C. Timo-Iaria. Limbic cholinergic pathways involved in sleep and emotional behavior. *Expl. Neurol.* **8:** 93–111, 1963.
8. Lomax, P. and D. J. Jenden. Hypothermia following systemic and intracerebral injection of oxotremorine in the rat. *Int. J. Neuropharm.* **5:** 353–359, 1966.
9. Miller, N. E., K. S. Gottesmann and N. Emery. Dose response to carbachol and norepinephrine in rat hypothalamus. *Am. J. Physiol.* **206:** 1384–1388, 1964.
10. Miller, N. E. Chemical coding of behavior in the brain. *Science* **148:** 328–338, 1965.
11. Papez, J. W. Proposed mechanism of emotion. *Archs. Neurol. Psychiat., Chicago* **38:** 725–743, 1937.
12. Stein, L. and J. Seifter. Muscarinic synapses in the hypothalamus. *Am. J. Physiol.* **202:** 751–756, 1962.