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CENTRAL ADMINISTRATION OF NEUROPEPTIDE Y INDUCES HYPOTHERMIA IN MICE. POSSIBLE INTERACTION WITH CENTRAL NORADRENERGIC SYSTEMS.

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Summary

Neuropeptide Y (0.24 and 1.17 nmol icv) and clonidine (0.025, 0.05 and 0.1 mg/Kg ip) induced a slight decrease of short duration of the rectal temperature in mice in a dose-dependent manner. While pretreatment with yohimbine (0.5 mg/Kg sc), was without effect on neuropeptide Y-induced hypothermia, it attenuated the hypothermic effect of clonidine. The association of neuropeptide Y (0.05 and 0.24 nmol icv) with clonidine (0.0125, 0.025, 0.05 and 0.1 mg/Kg ip) induced a synergistic effect, but it only was significant when neuropeptide Y 0.05 and 0.24 nmol icv was associated with clonidine 0.1 mg/Kg ip and when neuropeptide Y 0.05 nmol icv was associated with clonidine 0.05 mg/Kg ip. These results suggest that the effect of neuropeptide Y is not mediated by an interaction on alpha$_2$-adrenoceptor, but in accordance with these results, the existence of a collaborative mechanism between both neuropeptide Yergic and noradrenergic systems cannot be ruled out.

In 1982, TATEMOTO (1) isolated and sequenced a new peptide. The most abundant aminoacid residue found in the peptide was tyrosine, as a result of which the peptide was called neuropeptide Y (NPY). NPY is a 36 aminoacid peptide which occurs in very high concentrations in the CNS. It is the most abundant peptide in mammals CNS (2) where it is widely distributed, and after central administration produces a number of effects such as modulation of food intake (3,4,5,6), modulation of luteinizing hormone release (7) and modulation of memory (8). NPY has also been reported to produce a mild effect on temperature (9) and NPY has been shown to produce a mild hypothermia in dogs (10).

NPY is localized in hypothalamic nucleus, septum, striatum and pons (11),structures that have been implicated in the control of body temperature (12). In hypothalamus there is a concordance between noradrenaline (NA) and NPY terminal, but the hypothalamic NPY innervation arises largely from neurons which produce only NPY rather than from neurons in which NA and NPY coexist (13).

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However, there is a parallel distribution between NPY and NA, and it has been published that NPY cooperate with noradrenergic transmission in some localizations (14,15,16,17,18,19,20,21,22) and that noradrenergic systems may be implicated in thermal regulation (23), so it could be supposed that NPY co-operates with noradrenergic systems to modulate body temperature.

We have examined the effect of central NPY on temperature and the possible interaction between NPY and noradrenergic systems

Material and Methods

General procedure: Male albino mice OF1 (18-25 g.) from our University Reproduction Laboratories were used. The mice were housed in conventional cages and were kept on 12/12 h. light/dark cycle with the ligth phase from 8:00 a.m. to 8:00 p.m. They were allowed free access to food and water. Room temperature was controlled at 21±1 °C. Experiments were initiated at 4:00 p.m.

Rectal temperature (T) was measured using an analog thermometer at an ambient temperature of 21±1 °C. The thermometer rod was introduced 2 cm. into rectum. Before the administration of any drug, basal temperature (Tb) of each mouse was measured.

Drugs: Yohimbine (YHB) was obtained from Sigma Chemical Company. Dose used was 0.5 mg/Kg. It was administered subcutaneously (sc) 45 min. before the first T measurement (T0). This dose was chosen as a result of dose-effect studies carried out in our laboratories which have shown it to be one with no "per se" effect and which in our experimental conditions has been shown to prevent the effect of clonidine.

Clonidine (CND) was obtained from the Sigma Chemical Company. Doses used were: 0.0125, 0.025, 0.05 and 0.1 mg/Kg. It was administered ip. 30 min. before T0.

NPY (porcine) was obtained from Cambridge Biochemical Research Doses used were 0.05, 0.24 and 1.17 nmol. It was injected (icv) 10 min. before T0.

The icv injections were performed according to HALEY and McCORMICK (24) using a volume of 0.005 ml.

The ip and sc injections were performed using a volume of 10 ml/Kg.

T was measured 30, 60 and 90 min. after T0 (T1, T2 and T3 respectively).

Experimental protocol: Two hours before T0 determination, food was removed. After the Tb was determinated in each animal the effect of the following treatments combinations were evaluated

a) Saline (SS) (sc) followed 15 min. later by ip administration of SS, followed 20 min. later by icv administration of SS or NPY (0.05, 0.24 and 1.17 nmol). For n see Table I.

b) SS (sc) followed 15 min later by ip administration of CND (0.0125, 0.025, 0.05 and 0.1 mg/Kg) followed 20 min. later by

icv administration of SS. For n see Table I.

c) SS (sc) followed 15 min. later by ip administration of CND (0.0125, 0.025, 0.05 and 0.1 mg/Kg) followed 20 min. later by icv administration of NPY (0.05 or 0.24 nmol). For n see Table I.

d) YHB (0.5 mg/Kg sc) followed 15 min later by ip administration of CND (0.025, 0.05 and 0.1 mg/Kg) or SS followed 20 min. later by icv administration of SS. For n see Table I.

e) YHB (0.5 mg/Kg sc) followed 15 min. later by ip administration of SS, followed 20 min. later by icv administration of NPY (0.24 and 1.17 nmol). For n see Table I.

Statistical analyses: Changes in mean T from basal values are reported. The statistical analyses of results were evaluated by Mann-Whitney U-test or Kruskal-Wallis ANOVA followed when necessary by Mann-Whitney U-test.

Results

a) Effect of icv administration of NPY on T: NPY induced a decrease of T in a dose-dependent manner 10 min. after administration (T0) ($H=12.31$, $p < 0.01$). This effect was significant at doses of 0.24 and 1.17 nmol of NPY ($U=52$, $p<0.05$ and $U=38.5$, $p<0.01$ respectively) compared to control group. Table I.

b) Effect of ip administration of CND on T: Treatment with CND induced a dose-dependent hypothermic effect. This effect was significant at T0 ($H=26.98$, $p<0.0005$) because of the doses of 0.025 ($U=46.5$, $p<0.01$), 0.05 ($U=26.1$, $p<0.01$) and 0.1 mg/Kg ($U=2$, $p<0.001$) compared to control group. This hypothermic effect of CND was also significant at T1 ($H=16.7$, $p<0.005$) because of the dose of 0.1 mg/Kg ($U=17.5$, $p<0.001$). Table I.

c) Effect of sc pretreatment with YHB on the effect of NPY on T: The pretreatment with YHB (0.5 mg/Kg sc) did not induce any significant modification of the effect of NPY (0.24 and 1.17 nmol icv). Table I.

d) Effect of sc pretreatment with YHB on the effect of CND on T: The pretreatment with YHB (0.5 mg/Kg sc) prevented the effect of CND at 0.025 ($U=19$, $p<0.01$), 0.05 ($U=7$, $p<0.01$) and 0.1 mg/Kg ($U=10$, $p<0.01$)(p values compared with CND alone). This effect is shown in all the indicated doses at T0, and also for 0.1 mg/Kg ip ($U=7.5$, $p<0.001$) at T1. Table I.

e) Interaction between CND and NPY: The association of CND and NPY induced, constantly, a higher hypothermia -at T0- than CND or NPY alone. Similar results are shown at T1 when CND was administered at 0.1 mg/Kg. However this higher hypothermia was only significant when CND 0.1 mg/Kg with NPY 0.05 nmol. ($U=17$, $p<0.05$) or NPY 0.24 nmol. ($U=6.5$, $p<0.01$) and CND 0.05 mg/Kg with NPY 0.05 nmol. ($U=38$, $p<0.05$) were combined (p values were obtained at T0). Table I.

Discussion

The present paper has been approached with the purpose of

TABLE I

Influence of NPY (nmol), CND (mg/Kg), YHB (mg/Kg) and combined treatments on rectal temperature in mice.

Treatment	n	T0	T1	T2	T3
Saline	14	-0.31±0.15	-0.01±0.11	-0.24±0.15	-0.01±0.15
NPY 0.05	14	-0.22±0.12	0.07±0.16	-0.17±0.16	-0.01±0.19
NPY 0.24	14	-0.74±0.12(a)	-0.03±0.11	-0.13±0.12	-0.34±0.09
NPY 1.17	14	-1.03±0.17(b)	-0.36±0.24	-0.44±0.18	-0.11±0.11
CND 0.0125	16	-0.72±0.23	-0.06±0.12	0.13±0.11	0.10±0.10
CND 0.025	14	-1.05±0.24(b)	-0.13±0.16	-0.01±0.13	-0.12±0.19
CND 0.05	14	-1.19±0.20(b)	-0.34±0.27	0.14±0.24	-0.02±0.18
CND 0.1	14	-2.01±0.16(c)	-0.94±0.17(c)	-0.16±-.19	0.01±0.15
YHB 0.5	9	-0.28±0.14	0.00±0.10	-0.04±0.15	-0.13±0.15
YHB 0.5 + NPY 0.24	8	-0.74±0.22(a)	0.08±0.23	0.12±0.17	-0.18±0.16
YHB 0.5 + NPY 1.17	8	-1.10±0.20(b)	-0.50±0.18	-0.01±0.18	-0.39±0.17
YHB 0.5 + CND 0.025	8	-0.10±0.16(d)	0.12±0.12	-0.01±0.11	0.18±0.15
YHB 0.5 + CND 0.05	8	-0.15±0.12(e)	0.15±020	-0.04±0.07	0.25±0.14
YHB 0.5 + CND 0.1	7	-0.97±0.21(cd)	0.04±0.17(e)	-0.16±0.18	-0.06±0.13
CND 0.0125 + NPY 0.05	7	-0.71±0.25	-0.24±0.24	0.19±0.21	0.26±0.18
CND 0.025 + NPY 0.05	7	-1.24±0.20(b)	-0.13±0.24	0.11±0.19	0.11±0.20
CND 0.05 + NPY 0.05	13	-1.90±0.25(cf)	-0.31±0.21	0.05±0.15	-0.03±0.24
CND 0.1 + NPY 0.05	6	-3.28±3.31(cf)	-1.17±0.44(a)	-0.23±0.31	-0.02±0.28
CND 0.0125 + NPY 0.24	7	-1.26±0.23(b)	0.19±0.09	-0.27±0.24	0.26±0.13
CND 0.025 + NPY 0.24	14	-1.55±1.26(c)	0.19±0.17	0.17±0.16	0.28±0.16
CND 0.05 + NPY 0.24	14	-1.71±0.21(c)	-0.25±0.24	-0.14±0.19	0.11±0.19
CND 0.1 + NPY 0.24	6	-3.17±0.26(cd)	-1.63±0.33(b)	-0.73±0.27	-0.70±0.34

a=$p<0.05$ versus saline; b=$p<0.01$ versus saline; c=$p<0.001$ versus saline; d=$p<0.01$ versus CND; e=$p<0.001$ versus CND; f=$p<0.05$ versus CND

establishing whether NPY could induce some effect on rectal temperature in mice. A possible interaction with central noradrenergic systems was looked for.

The central distribution of NPY, with high density of peptidergic fibers -containing NPY- in hypothalamus, septum, pons and striatum (11),structures which are linked to the control of body temperature (12) may suggest that NPY could be involved in thermoregulation MOIKA et al.(10) have shown a hypothermic effect of NPY in dogs, and GRAY and MORLEY (9) have reported a similar effect but in an irregular manner, in rats.

In the present paper we report that icv administration of NPY slightly decreases the rectal temperature in mice and for a short period. This NPY hypothermic effect could be mediated by a direct interaction between NPY and its own specific receptors (25,26,27) but it could also be mediated by a modulatory effect of NPY on other neurotransmission systems. In fact, NPY and NA are distributed in hypothalamus, but it cannot be stated that both NPY and NA are found in the same neurons in hypothalamus (13). Nevertheless, several papers have been published showing a functional interaction between both NPY and NA systems in some hypothalamus-mediated effects such as control of ingestive behavior (3,4,6,7). Moreover, it has been published that NPY modulates the release of NA in hypothalamus by an $alpha_2$-adrenoceptor-mediated mechanism (28). In fact, several papers show a possible modulatory action of NPY on $alpha_2$-presynaptic adrenoceptors. It has also been published that NPY "in vitro" selectively increases the number of $alpha_2$-adrenergic binding sites in membranes of the medulla oblongata of the rat (14) and also inhibits forskolin-induced accumulation of cAMP (29,30,31) in a manner similar to CND. Likewise the NPY depresses the secretion of NA induced by field stimulation (15,28) and it has been published that NPY induced a suppression of activity in the rat. This effect is prevented by pretreatment with $alpha_2$-adrenoceptor antagonist (32). However, the results presented in this paper show that the hypothermic effect of NPY may not be mediated by a modulatory action of NPY on $alpha_2$-adrenoceptor, since pretreatment with the $alpha_2$-antagonist YHB, injected at a dose that prevented the hypothermic effect of CND, did not prevent the hypothermic effect of NPY. Nevertheless, we cannot rule out the hypothesis of a functional collaboration between NPY and noradrenergic systems since the combined treatment of CND and NPY always induced a synergistic effect although it was only sometimes significant (see results). According to the results, it seems evident that on the contrary to other NPY effects which seem to be mediated through $alpha_2$-adrenoceptor, the NPY hypothermic effect is not mediated through such a receptor. Moreover the existence of a mechanism of collaboration between NPY fibers and noradrenergic systems which control the rectal temperature in mice seems evident Nevertheless, the existence of other more profund effects of NPY which mediate the temperature changes cannot be ruled out.

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References

1. K.TATEMOTO, Systemic Role of Regulatory Peptides, S.R.Bloom,

J.M.Polak and E.Morley. Lindenlaub (Eds), p.507-529, F.K. Achattaver Verlag. Stuttgart-New York (1982).
2. T.E.ADRIAN, J.M.ALLEN, S.R.BLOOM, M.A.GHATEI, M.N.ROSSOR, G.W. ROBERTS, T.J.CROW, K.TATEMOTO and J.M.POLAK, Nature, 306 584-586 (1983).
3. B.G.STANLEY, S.E.KYRKOULI, S.LAMPERT and S.F.LEIBOWITZ, Peptides 7 1189-1192 (1986).
4. J.E.MORLEY, E.N.HERNANDEZ and J.F.FLOOD, Am.J.Physiol. 253 R516-R522 (1987).
5. W.J.KUENZEL, L.W.DOUGLASS and B.A.DAVISON, Peptides 8 823-829 (1987).
6. J.T.CLARK, R.S.GIST, S.P.KALRA and P.S.KALRA, Physiol.Behav. 43 417-423 (1988).
7. L.G.ALLEN, P.S.KALRA, W.R.CROWLEY and S.P.KALRA, Life Sci. 37 617-623 (1985).
8. J.F.FLOOD, E.N.HERNANDEZ and J.E.MORLEY, Brain Res. 421 280-290 (1987).
9. T.S.GRAY and J.E.MORLEY, Life Sci. 38 389-401 (1986).
10. H.MOIKA, A.INUITZ, M.O.OOYA, M.SUENAGA, N.MIZUNO and S.BABA, Digest. Dis. Sci. 29 555 (1984).
11. M.E.DE QUIDT and P.C.EMSON, Neuroscience 19 1011-1023 (1986).
12. R.NECKER, Thermal Physiology, J.R.S.Hales (Ed), p.53-61, Raven Press, New York (1984).
13. E.L.GUSTAFSON and R.Y.MOORE, Neurosci. Lett. 83 53-58 (1987).
14. L.F.AGNATI, K.FUXE, F.BENFENATI, N.BATTISTINI, A.HARFSTRAND, K.TATEMOTO, T.HOKFELT and V.MUTT, Acta Physiol.Scand 118 293-295 (1983).
15. J.M.LUNDBERG and L.STJARNE, Acta Physiol.Scand. 120 477-479 (1984)
16. J.M.LUNDBERG, Neurosci.Lett. 26 S209 (1986).
17. L.STJARNE and J.M.LUNDBERG, Prog.Brain.Res. 68 263-278 (1986)
18. L.STJARNE, J.M.LUNDBERG and P.ASTRAND, Neuroscience 18 151-166 (1986).
19. M.STJERNQUIST, CH.OWMAN, N.O.SJORGER and F.SUNDLE, Biol. Reprod. 36 149-155 (1987).
20. R.N.DALY and J.P.HIEBLE, Eur.J.Pharmacol. 138 445-446 (1987)
21. T.HOKFELT, J.M.LUNDBERG, T.TATEMOTO, V.MUTT, L.TERENIUS, J.POLAK, S.BLOOM, C.SASEK, R.ELDE and M.GOLDSTEIN, Acta Physiol. Scand. 117 315-318 (1983).
22. L.KASAKOV, J.ELLIS, K.KIRKPATRIK, P.MILNER and G.BURNSTOCK, J. Aut. Nerv. Syst. 22 75-83 (1988).
23. E.ZACNY, J. Pharm. Pharmac. 34 455-456 (1982).
24. T.J.HALLEY and W.G.McCORMICK, Br.J.Pharmacol. 12 12 (1957).
25. A.SARIA, E.THEODORSSON-NORHEIM and J.M.LUNDBERG, Eur. J. Pharmacol. 107 105-107 (1985).
26. A.SARIA, G.SKOFITSCH, A.HEMSEN, E.THEODORSSON-NORHEIM and J.M.LUNDBERG, Neurosci. Lett.26 S221 (1986).
27. C.WAHLESTEDT, L.EDVINSSON, E.EKBLAD and J.HAKANSON, Neuronal Messengers in Vascular Function, A. Nobin and Ch. Owman (Eds), p 123, Fernstrom Symp, n 10 (1987).
28. H.YOKOO, D.H.SCHLESINGER and M.GOLDSTEIN, Eur. J. Pharmacol., 143 283-286 (1987).
29. S.PETRENKO, M.C.OLIANAS, P.ONALI and G.L.GESSA, Eur. J. Pharmacol. 136 425-428 (1987).
30. B.B.FREDHOLM, I.JANSEN and L.EDVINSSON, Acta Physiol. Scand 124 467-471 (1985).
31. J.HAGGBLAD and B.B.FREDHOLM, Neurosci.Lett. 82 211-216 (1987).
32. M.HEILIG, C.WAHLESTEDT and E.WIDERLOV, Eur. J. Pharmacol. 157 205-215 (1988).